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The relationship between faecal haemoglobin concentration and risk of significant colorectal neoplasia in screening and symptomatic populations.

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List of abbreviations and explanatory notes

Abbreviation	Whole Phrase	Explanatory note
AN	Advanced neoplasia	Neoplasia which is either cancer or higher-risk adenoma.
BMI	Body Mass Index	Value calculated as weight in kilograms divided by the square of height in metres.
CI	Confidence Intervals	Range of values that the population variable would lie within if repeated samples were taken, with a specified probability.
CLSI	Clinical and Laboratory Standards Institute	Organisation facilitating the development and implementation of clinical laboratory testing standards.
CRC	Colorectal cancer	Development of cancer in the colon or rectum, also known as bowel cancer. Includes colorectal polyp cancers.
f-Hb	Faecal haemoglobin concentration	Concentration of haemoglobin detected in a sample of faeces.
FIT	Faecal immunochemical test for haemoglobin	Test for presence of haemoglobin in faeces that use labelled antibodies specific for binding to the intact globin moiety of human haemoglobin to allow measurement of the bound complex by a variety of techniques.
gFOBT	Guaiac faecal occult blood test	Test for haemoglobin in faeces, using a card with an integral guaiac impregnated paper that, following development with hydrogen peroxide, detects the presence of the haem component of haemoglobin through detection of blue colour.
GP	General Practitioner	Medical doctor who treats acute and chronic illnesses and provides preventative care to people in their local community.

Hb	Haemoglobin	Oxygen-carrying molecule in red blood cells.
HGD	High grade dysplasia	Loss of normal differentiation of epithelium with a more advanced progression towards malignant transformation.
HIS	Healthcare Improvement Scotland	National healthcare improvement organisation as part of the Scottish National Health Service. Work includes developing evidence-based guidance and standards for clinical practice through SIGN, supporting improvement of healthcare practice and reporting on healthcare performance.
HRA	Higher-risk adenoma	Defined as ≥3 adenomas, or any adenoma with a maximum diameter ≥10 mm, taken from recommendation from the British Society of Gastroenterology (Atkin & Saunders, 2002) as used in Scotland.
IC	Interval cancer	Colorectal cancer diagnosed after a screening test or examination in which no cancer is detected and before the date of the next recommended examination.
IBD	Inflammatory Bowel Disease	Group of inflammatory conditions of the colon and small intestine, principally Crohn's disease and ulcerative colitis.
IQR	Interquartile range	The difference between the lower quartile and the upper quartile of the data, ignoring extreme values.
ISO	International Organization for Standardization	Independent, non-governmental organisation with 162 member countries, developing voluntary international standards covering almost every industry.
IT	Information technology	The use of computers and telecommunications equipment to store, retrieve, transmit and manipulate data.
LGD	Low grade dysplasia	Represents loss of normal differentiation of normal epithelium with a low risk of progression towards high risk dysplasia and malignant transformation.

LRA	Low-risk adenoma	Defined as <3 adenomas and with a maximum diameter <10 mm, taken from recommendation from the British Society of Gastroenterology (Atkin & Saunders, 2002) as used in Scotland.
NICE	National Institute for Health and Care Excellence	Public body of Department of Health in the United Kingdom, serving England and Wales, that publishes guidance as to the most appropriate treatment regime for a range of different diseases.
NPV	Negative Predictive Value	Negative Predictive Value (NPV) is the proportion of participants with a negative test who are, in fact, free from the disease in question e.g. correctly predicted as not having colorectal cancer.
PPV	Positive Predictive Value	Positive Predictive Value (PPV) in colorectal cancer screening is the proportion of participants undergoing colonoscopy as follow-up to a positive test who actually have disease detected.
ROC	Receiver operating characteristic	A ROC curve is a graphical plot of the true positive rate against the false positive rate for different cut-off concentrations.
SCD	Significant Colorectal Disease	Term to encompass colorectal cancer, higher-risk adenoma and inflammatory bowel disease.
sFOBT	Sensitive guaiac faecal occult blood test	High-sensitivity (low analytical detection limit) guaiac faecal occult blood test.
SIGN	Scottish Intercollegiate Guidelines Network	Multidisciplinary working groups who develop and disseminate evidence-based clinical practice guidelines. Scottish counterpart of NICE.
SIMD	Scottish Index of Multiple Deprivation	SIMD ranks small areas from the most deprived (ranked 1) to the least deprived (ranked 6,505) according to income, employment, health, education, geographic access, crime and housing.

Further explanatory notes

Adenoma: Benign epithelial tumour which in some cases will progress towards malignancy as the known precursor of colorectal cancer.

Colonoscopy: Endoscopic examination of the colorectum.

Distal (location of lesion): Refers to region of colon beyond the splenic flexure.

Dukes' stage: System devised by Cuthbert Dukes for the staging of colorectal cancers. Dukes' A describes cancer contained within the bowel wall; Dukes' B describes cancer with invasion into the muscle layer of the bowel wall but with no lymph node involvement; Dukes' C describes cancer involving lymph nodes; Dukes' D describes cancer with widespread metastases.

Dysplasia: Loss of normal differentiation of epithelium.

Hyperplastic polyp: A commonly occurring benign cluster of cells projecting as a growth from the mucosal lining of the colorectum.

Malignant: Refers to a tumour that is capable of spreading to adjacent and distant tissues.

Mean: Sum of a set of numbers, divided by the number of numbers in the set.

Median: The value separating the lower half of numbers in the set from the upper half of numbers in the set.

Neoplasia: Abnormal growth of tissue.

Proximal (location of lesion): Refers to region of colon from the caecum up to and including the splenic flexure.

p-value: Probability of obtaining the observed results, used for testing a statistical hypothesis.

Quintile: Any one of five equal groups into which data have been divided.

Sensitivity: Sensitivity is a measure of the test's ability to identify the disease being screened for, i.e. the percentage of participants in the screened population who have colorectal cancer, who are identified with a positive screening test result.

Specificity: Test specificity is a measure of the test's ability to rule out those without disease, i.e. the percentage of participants in the screened population who do not have colorectal cancer, who are identified with a negative screening test result

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Declaration

I declare that I am the author of this thesis and it is a record of work carried out by myself and it has not been previously accepted for a higher degree. I have consulted all references cited.

Signature

Date

Name

This is to certify that the candidate has fulfilled the conditions of the regulations appropriate of the degree of Doctor of Philosophy in the University of Dundee and that the candidate is qualified to submit this thesis in application for that degree.

Signature

Date

Name

Summary

Faecal immunochemical tests for haemoglobin (FIT) are replacing traditional guaiac-based faecal occult blood (gFOBT) tests in bowel screening programmes due to their many advantages. An evaluation of using quantitative FIT within the Scottish Bowel Screening Programme (SBSP) has taken place with faecal haemoglobin (Hb) concentration recorded for 38,720 participants. Subsequently, it has been established that faecal Hb concentration is related to severity of colorectal neoplastic disease, with higher median faecal Hb concentration in participants with advanced neoplasia compared to those with less severe outcomes (200.0 v. 166.0 µg Hb/g faeces, $p < 0.0001$). Those with elevated faecal Hb concentration (60.0 - 79.9 µg Hb/g faeces) at the time of a negative test result (< 80.0 µg Hb/g faeces) were more likely to be later diagnosed with an interval cancer than those with undetectable Hb (adjusted odds ratio = 24.7, 95% CI: 4.9 - 124.6). Follow-up of participants with a negative test result then testing positive in the subsequent screening round allowed calculation of an adjusted odds ratio of 38.0 (95% CI: 20.2 – 71.2) for advanced neoplasia in those with initial faecal Hb concentration 60.0 - 79.9 µg Hb/g faeces compared to those with faecal Hb concentration < 20.0 µg Hb/g faeces. These results give firm support to the role of faecal Hb concentration as a strong predictor of future risk of advanced neoplasia. The use of FIT in symptomatic patients was also evaluated, with results showing that using a cut-off faecal Hb concentration of any detectable Hb would have ruled out colorectal cancer and could have reduced the referral rate by 40%. With introduction of FIT now approved for the SBSP, it is hoped that a risk scoring system can be developed based on age, gender and faecal Hb concentration to better direct colonoscopy resource and reduce the proportion of interval cancers.

Publications

This thesis contains work that has resulted in the following publications:

Fraser, C. G., **Digby, J.**, McDonald, P. J., Strachan, J. A., Carey, F. A. & Steele, R. J. C. (2012) Experience with a two-tier reflex gFOBT/FIT strategy in a national bowel screening programme. *J Med Screen, 19*(1), 8-13.

Digby, J., Fraser, C. G., Carey, F. A., McDonald, P. J., Strachan, J. A., Diament, R, H., Balsitis, M. & Steele, R. J. C. (2013) Faecal haemoglobin concentration is related to severity of colorectal neoplasia. *J Clin Pathol, 66*(5), 415-419.

Digby, J., McDonald, P. J., Strachan, J. A., Libby, G., Steele, R. J. C. & Fraser, C. G. (2014) Deprivation and faecal haemoglobin concentration: implications for bowel cancer screening. *J Med Screen, 20*(2), 80-85.

Mowat, C., **Digby, J.**, Strachan, J. A., Wilson, R., Carey, F. A., Fraser, C. G. & Steele, R. J. C. (2015) Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel symptoms. *Gut*. Published Online First: doi:10.1136/gutjnl-2015-309579. [Epub ahead of print]

Digby, J., Fraser, C. G., Carey, F. A., Lang, J., Stanners, G. & Steele, R. J. C. (2015) Interval cancers using a quantitative faecal immunochemical test for haemoglobin (FIT) when colonoscopy capacity is limited. *J Med Screen*. pii: 0969141315609634. [Epub ahead of print]

Other publications relevant to parts of the work described in this thesis:

McDonald, P. J., Strachan, J. A., **Digby, J.**, Steele, R. J, C. & Fraser, C. G. (2011) Faecal haemoglobin concentrations by gender and age: implications for population-based screening for colorectal cancer. *Clin Chem Lab Med, 7*;50(5), 935-940.

Steele, R. J. C., McDonald, P.J., **Digby, J.**, Brownlee, L., Strachan, J. A., Libby, G., McClements, P. L., Birrell, J., Carey, F. A., Diament, R. H., Balsitis, M. & Fraser, C. G. (2013) Clinical outcomes using a faecal immunochemical test for haemoglobin as a first-line test in a national programme constrained by colonoscopy capacity. *United European Gastroenterol J, 1*(3), 198-205.

Digby, J., McDonald, P. J., Strachan, J. A., Libby, G., Steele, R. J. C. & Fraser, C. G. (2013) Use of a faecal immunochemical test narrows current gaps in uptake for sex, age and deprivation in a bowel cancer screening programme. *J Med Screen, 20*(2):80-85.

1. Introduction

1.1 The problem and the basis for colorectal cancer screening

Colorectal cancer presents a significant health problem in Scotland, where it is the third most commonly diagnosed cancer in both men and women and accounts for the second highest number of cancer deaths after lung cancer. The most recent data available from the Scottish Cancer Registry (Information Services Division (ISD) Scotland, 2015a) report over 3,800 cases diagnosed in 2013, representing a higher rate of the disease than seen in most other Western countries and resulting in almost 1,600 deaths per year. The risk of developing colorectal cancer increases with age, with 95.0% of cases diagnosed in Scotland between 2009 and 2013 occurring in people over the age of 50 years. Scottish data from the same time period show risk of developing colorectal cancer at 0.9%, or 1 in 113, up to the age of 64 years, rising to an overall lifetime risk of 5.9%, or 1 in 17. The overall five-year survival rates, relative to the general population, are around 60% for both genders, with prognosis highly dependent on early detection and treatment. The latest colorectal cancer statistics from Cancer Research UK (2015) report that only around 9% of patients in the UK are diagnosed at the earliest stage of the disease, classified as Dukes' stage A, where the cancer has not yet penetrated through the bowel wall. Five-year relative survival rate at this stage is reported at over 93%, dropping progressively through to the late stage Dukes' D involving widespread metastases, when only 6.6% of patients survive five years or more.

Numerous epidemiological, clinical, histopathological and genetic studies provide indirect support for the widely accepted view that most, if not all colorectal cancers arise from premalignant lesions, as summarised in the review by Leslie *et al*. (2002) This process follows a stepwise sequence from normal epithelium to the formation of adenomas which, over time, exhibit varying degrees of dysplasia. Adenomas are highly prevalent among the general population, occurring in around 40% of inhabitants of Western countries, although only around 3% go on to develop colorectal cancer. Furthermore, a long precancerous phase of around 10-15 years exists in most cases. (Muto *et al.*, 1975) Malignant transformation is associated with adenoma size, (Villavicencio & Rex, 2000) but it is impossible to predict which adenomas will eventually develop into cancer. It is therefore recommended that adenomas are removed if possible, by polypectomy at colonoscopy, or else surgically for particularly large or flat lesions for which endoscopic removal may carry a high risk of excessive bleeding or perforation.

The high prevalence of potential premalignant lesions in the general population, along with the fact that symptoms associated with colorectal cancer do not occur until the later stages of the disease, and the clear importance of early detection in terms in survival rates contribute to the status of colorectal cancer as an ideal candidate for population screening. Criteria for the appraisal of the validity of a screening programme were described by Wilson & Jungner (1968) for the World Health Organisation. Based on these classic principles, The United Kingdom National Screening Committee developed updated criteria (Public Health England, 2013), listed under five subheadings: 1) the condition; 2) the test; 3) the intervention; 4) the screening programme and 5) implementation criteria. To summarise, the condition being screened for should be a significant health problem, an appropriate and valid screening tool should be available with an agreed cut-off and policy for follow-up of

those with positive test results. An effective intervention that shows association with better outcomes than usual care and the screening programme should be supported by evidence from randomised controlled trials (RCT) of a reduction in mortality and morbidity. The benefits of screening should outweigh any harms arising from overdiagnosis, false screening results and complications. The screening process as a whole should be cost-effective. Finally, several implementation criteria are listed including consideration of current interventions, providing informed choice to invitees and scientific justification of decisions on eligibility, screening interval length and test sensitivity. Colorectal cancer screening programmes are now employed in many countries with the main principles being early detection of cancer and removal of its precursors to prevent progression into malignant lesions.

1.2 Screening tests for the presence of haemoglobin in faeces

Several methods of population colorectal cancer screening methods exist, each with associated advantages and drawbacks relating to various issues including sensitivity and specificity for screen-relevant neoplasia, uptake of screening, associated risks, costs and capacity of resources. Colonoscopy is regarded as the gold standard method of detecting colorectal cancer and its precursors, with Winawer *et al*. (1993) demonstrating that colonoscopic removal of all detected polyps resulted in reduced incidence of colorectal cancer. However, colonoscopy is expensive, invasive and associated with potential complications. As such, it is generally used in population screening programmes as part of a two-stage system, where abnormalities detected by the primary test are followed up with referral for colonoscopy as the reference test. The most commonly used strategy involves the use of tests in the first instance to detect the presence of haemoglobin (Hb) in faeces, commonly called faecal occult blood tests. The presence of Hb in faeces can be associated with various

gastrointestinal conditions, but particularly with colorectal cancer and larger precursor lesions.

Traditional guaiac faecal occult blood tests (gFOBT) are used in various countries. This test involves the application of faecal samples on to a test card with an integral guaiac impregnated paper. To develop the test, hydrogen peroxide is dropped onto the paper to detect traces of Hb indirectly, based on the oxidation of the guaiac by the peroxidase activity of the haem moiety of Hb to give blue or green colours. As yet, gFOBT is the only faecal test proven to reduce mortality in large-scale, long-term RCT using Hemoccult, a commonly used traditional gFOBT. Trials conducted between 1975-2002 in Minnesota, USA, (Mandel *et al.*, 1993) Nottingham, UK, (Hardcastle *et al.*, 1996) Funen, Denmark (Kronborg *et al.*, 1996) and Gothenberg, Sweden (Kewenter *et al.*, 1994) were examined in a meta-analysis by Towler *et al*. (1998) These studies involved around 330,000 participants aged between 45-80 years and follow-up ranged from 7.8-13.0 years. The meta-analysis demonstrated that gFOBT screening was associated with a 16% reduction in colorectal cancer mortality in those intended to be screened compared with those not invited for screening. This effect rose to a 23% reduction when adjusting for those actually screened. In addition, publication of outcomes of the Bowel Cancer Screening Programme in England following the first 1 million tests after its roll-out in 2006 reported that, if the early results are maintained, the programme will meet its aim of reducing colorectal cancer mortality by 16%. (Logan *et al.*, 2012) Seventy percent of cancers were early stage, meaning that a key aim of colorectal cancer screening that is, to detect cancer whilst death from colorectal cancer is still preventable, is being met. Updated work on the cohort involved in the Minnesota RCT has shown that colorectal cancer mortality decreased with both annual and biennial gFOBT screening over the 30 year follow-up period

(relative risk 0.68 [95% confidence interval [CI]: 0.56 – 0.82] and 0.78 [95% CI: 0.65 – 0.93], respectively), but with no decrease in all-cause mortality. (Shaukat *et al.*, 2013)

Debate exists, not just in colorectal cancer screening but with all mass population screening programmes, around the fact that there is a lack of evidence of a reduction in all-cause mortality in the literature. (Penston, 2011; Steele & Brewster, 2011). Potential sources of bias can be introduced when measuring disease-specific mortality that are not an issue with all-cause mortality which, by nature, simply counts the number of deaths, regardless of cause. It is argued that this bias leads to an overestimation of the benefits and an underestimation of the harms of screening. One example of the potential for harm in colorectal cancer screening comes from the complications associated with colonoscopy. Bowel perforation, although rare, will occur in some healthy individuals undergoing investigation following a positive screening test result and can, in some cases, lead to death. Other harms arising from the screening process are more difficult to quantify. Those with false negative screening test results may be subject to what is described as the “certificate of health effect”. This occurs when an individual receiving a negative result becomes less likely to improve their lifestyle to reduce disease risk and may also ignore disease symptoms. In addition, those with false positive results may be vulnerable to psychological morbidity arising from the anxiety of receiving a positive test result letter and while waiting for colonoscopy appointment. Another issue when screening screening for the early stages of disease is overdiagnosis. Some early stage colorectal cancers may never have been destined to become symptomatic in the lifetime of the screening participant, and the harms associated with treatment, potentially including death, means that these participants are therefore disadvantaged by their participation in screening. Although the use of all-cause mortality would eliminate these examples of bias towards the case for screening being effective, it has been argued that it is

unreasonable to insist that a screening programme must be supported by evidence of a reduction in this measure (Steele & Brewster, 2011). Colorectal cancer accounts for 3% of all deaths in the UK, meaning that the likely reason that no studies to date have demonstrated a reduction in all-cause mortality is that they have not been highly enough powered for this to be possible; an RCT of the required large sample size would be unfeasible. It is, however, important to continue to monitor the effect of screening on all-cause mortality to ensure no increase in this measure is occurring..

A matched cohort study performed using data from the three pilot rounds of the Scottish Bowel Screening Programme revealed a 10% decrease in relative risk of colorectal cancer mortality in those invited to take part in gFOBT screening compared with controls matched by age, gender and deprivation; this rose to 27% in participants. (Libby *et al.*, 2012)

Despite being proven to play a role in the reduction of colorectal cancer mortality and being relatively cheap and easy to post to participants, gFOBT also have disadvantages. Although limited literature exists on the subject, a major issue is that gFOBT are associated with a high interval cancer proportion, i.e., colorectal cancer arising between screening rounds following a negative result. Results from the RCT on gFOBT screening in Nottingham revealed an interval cancer proportion of 51.3%, (Hardcastle *et al.*, 1996), the Funen study 55.2% (Kronborg *et al.*, 1996) and, in a non-randomised trial of gFOBT effectiveness conducted in Burgundy, 59.3%. (Faivre *et al.*, 1991) Steele *et al*. (2012) investigated the proportion of interval cancer detected within the demonstration pilot of the Scottish Bowel Screening Programme from 2000-2007. Here, it was shown that the interval cancer proportion was 31.2%, 47.7% and 58.9% in the first, second and third rounds respectively. In France, Tazi *et al*. (1999) compared characteristics of interval cancers with gFOBT screen-detected cancers over five

rounds of screening. Of 398 colorectal cancers detected, 57.8% were interval cancers. These studies show that interval cancers are consistently accounting for more than half of the colorectal cancers detected in biennially screened populations, indicating poor test sensitivity. Methods to improve test sensitivity without an unacceptable associated fall in specificity may be aided by knowledge of factors showing association with interval cancers. Some characteristics have been identified from published studies analysing colorectal cancer diagnosed both following negative gFOBT results and negative colonoscopy.

In the investigations of Steele *et al*., (2012) 50.2% of interval cancers were found to arise in women, representing a significantly higher proportion than seen with screen-detected cancers (35.5%) and in the non-screened population (40.6%). In addition, significantly more interval cancers were situated in the right colon or rectum than in both the screen-detected group and the non-screened group. Assuming the cancers were present at the time of the negative gFOBT, these findings suggest that gFOBT may tend to preferentially detect cancers in men and in the left side of the colon. Similar findings were demonstrated by Gill *et al*. (2012) using colorectal cancer audit data from the North-East of England. The proportion of cancers occurring in women was higher in those with an interval cancer (39.6%, compared with 27.0% among screen-detected cancers), again suggesting that women may be disadvantaged with gFOBT. Also, a third of interval cancers were located proximally compared with just over a fifth in the screen-detected group, suggesting that gFOBT is also less effective for detecting right-sided lesions. On the other hand, Tazi *et al*. (1999) found that, amongst cancers of the rectum, 72.2% were interval cancers, a higher proportion than at any other site. Also, less interval cancers were classified as earlier stage than screen-detected cancers (57.4% compared with 73.8%). These results are consistent with those of Jensen *et al*., (1992) who found interval cancers in their study group to be

more advanced, larger, less often Dukes' stage A, more invasive to neighbouring organs and less often able to be resected for cure. Again, interval cancers were located more often in the rectum than cancer detected through screening and those in non-responders to screening. The reasons for the apparent association of colorectal cancer in the right colon and rectum with interval cancers in screening with tests for the presence of Hb in faeces are not clear and this requires further attention. Some potential explanations, however, include a greater degree of degradation of Hb arising from right-sided lesions due to a longer passage through the colon, non-haemolysed erythrocytes from rectal tumours not yielding a positive screening test result and rectal tumours being associated with faster growth rates. (Launoy *et al.* 1997)

Further evidence of characteristics associated with cancers missed by a screening test comes from studies of cancer diagnosed following negative colonoscopy. Brenner *et al*. (2012) conducted a case-control study to assess predictors of colorectal cancer occurring within 10 years of a colonoscopy where no lesion was detected. They found that a significantly higher proportion of these so-called interval cancers were found in women than in the population with screen-detected cancers, (56.4% v. 33.7%); female gender showed an odds ratio for interval cancer of 2.28 (95% CI: 1.35 - 3.83). Proximal location in the colon was also independently associated with interval cancer, with an odds ratio of 1.98 (95% CI: 1.17 - 3.35). A positive gFOBT result was the most common indication for the negative colonoscopy and colonoscopy was more often incomplete amongst interval cancer cases than controls. Interestingly, the association of these colonoscopy-related factors with interval cancer was gender-specific; follow-up of a positive gFOBT result was strongly associated with negative colonoscopy preceding colorectal cancer in men, but not in women, whereas incompleteness of colonoscopy showed strong association with interval cancer in women, but with no association found in men. The higher proportion of interval cancers located proximally

compared with screen-detected colorectal cancer was only statistically significant for the first three years following negative colonoscopy. Furthermore, interval cancers were more commonly late stage than screen-detected cancers and this was particularly true for proximal cancers and cancers detected within three years of a negative colonoscopy. This evidence points towards interval cancers being previously missed lesions rather than fast-growing, *de novo* tumours not present at the time of colonoscopy. Conversely, interval cancers occurring with a positive gFOBT result as the indicator for negative colonoscopy were associated with distal location in the colon. The authors speculated that, with sensitivity for adenoma detection with gFOBT being higher for men than women, small adenomas detected with gFOBT were being missed by colonoscopy in men. Interestingly, a study by Shaukat *et al*.(2010) examining gene mutations associated with interval cancer looked only at cancers diagnosed within five years of a *complete* colonoscopy, meaning that failure to inspect the proximal region of the colon was less of an issue. A multivariate logistic regression model showed proximal location in the colon and micro-satellite instability to be independently associated with interval cancer (odds ratio = 1.85, 95% CI: 1.01 – 3.8 and 2.7, 95% CI: 1.08 – 6.8, respectively). Several other studies have also identified an association between proximal location in the colon and interval cancer following negative colonoscopy. (Cooper *et al.*, 2012; Farrar *et al.*, 2006; Hosokawa *et al.*, 2003; Singh *et al.*, 2010; Singh *et al.*, 2006)

The high interval cancer proportion associated with gFOBT is of concern, with the poor test sensitivity suggested by these rates being a major disadvantage. Further research is required to identify potential methods of reducing proportions of interval cancer and addressing the imbalances seen between men and women, and between tumour sites, such as replacing gFOBT with a test that has higher clinical sensitivity.

Further to the high interval cancer proportion, most likely caused by false negative test results, gFOBT is also associated with a high false positive test result rate. Data from the Nottingham (Hardcastle *et al.*, 1996) and Funen (Kronborg *et al.*, 1996) RCT show that no neoplasia is detected in around half of colonoscopies performed following a positive gFOBT result. One reason for this may be that gFOBT are not specific for human Hb and are therefore subject to possible dietary interference, for example from red meat. In addition, gFOBT have also been thought to be susceptible to interference from dietary high-peroxidase vegetables contributing to a false positive test result, and conversely, citrus fruits and high-dose vitamin C can inhibit the peroxidase reaction, generating false negative test results. In consequence, some screening programmes instruct participants to adhere to dietary restrictions ahead of collection of faeces, although these restrictions may act as a barrier to screening and result in lower participation rates. (Pignone *et al.*, 2001) However, a systematic review by Konrad (2010) concluded that data from four RCT did not support dietary restrictions with gFOBT, with only large quantities of red meat having an effect, and recommended that restrictions are abandoned to improve uptake.

Several further disadvantages associated with gFOBT have been highlighted. (Duffy *et al.*, 2011; Fraser, 2008) Test interpretation is subjective, leaving the detection of subtle colour changes on the test card open to inter-observer variation. This testing is impossible to automate for more rapid-sample turnaround and as such, gFOBT have been regarded as inappropriate for large-scale population screening. Another weakness identified with gFOBT is that with guaiac being a tree bark extract, it is subject to batch-to-batch variation that can result in fluctuation in the test positivity rate. This is clearly a drawback in screening programmes looking to maintain a consistent rate of referrals to a limited colonoscopy resource.

Newer, faecal immunochemical tests for Hb (FIT) have become available and are increasingly being used in screening programmes due to the plethora of advantages they have over gFOBT. Unlike gFOBT, FIT are specific for the detection of human Hb, eliminating any potential for false positive test result arising from dietary interference. They differ from gFOBT in that they utilise antibodies to detect the globin moiety of the Hb molecule. The globin from Hb released in the upper gastrointestinal (GI) tract is broken down by digestive enzymes before reaching the large bowel, meaning that FIT are also more specific than gFOBT for lower GI bleeding. In addition to the ease-of-use advantage owing to the lack dietary restriction requirements, modern FIT generally provide a more convenient method of sample collection, using probes attached to the lid of the collection device rather than the less convenient card and spatula methods associated with gFOBT. Due to its greater sensitivity for detecting blood in the faeces, FIT are often one sample kits, in contrast to gFOBT, which usually require two samples of faeces from each of three consecutive bowel movements. These less-demanding features of sample collection are thought to account for the improvements in uptake seen when comparing FIT with gFOBT, widely reported across several RCTs (Federici *et al.*, 2005; Hoffman *et al.*, 2010; Hol *et al.*, 2010; Hol *et al.*, 2009) and other publications (Birkenfeld *et al.*, 2011; Canadian Agency for Drugs and Technologies in Health (CADTH), 2010; Digby *et al.*, 2013; Martínez *et al.*, 2011; Shuhaibar *et al.*, 2011; Vart *et al.*, 2012). A particularly pleasing outcome from the evaluation of quantitative FIT within the Scottish Bowel Screening Programme was that the improvement in uptake seen when comparing those invited with a group invited via the usual gFOBT/qualitative FIT two-tier reflex algorithm was greatest in men, those in the youngest age quintiles, and those in the most deprived quintiles of socioeconomic status. (Digby *et al.*, 2013) A thorough recent review of tests and investigations for colorectal cancer screening makes the statement that “the ideal screening test is the

test that gets done" and points towards FIT as the non-invasive test of choice for implementation in organised screening worldwide. (Carroll *et al.*, 2014)

Another major advantage of FIT is that automated versions are available that not only have advantages over gFOBT in terms of improving analytical quality and potentially laboratory costs, but also the quantitative nature of these tests mean that a faecal Hb concentration result is generated. This means that, rather than being limited to the cut-off faecal Hb concentration set by the manufacturer, quantitative FIT allow screening programme organisers to set an appropriate cut-off concentration for a positive test result to give optimum sensitivity and specificity within current colonoscopy capacity. Indeed, Terhaar sive Droste *et al*. (2011) demonstrated the benefits of different cut-off concentrations by showing substantial reductions in test positivity rates with increasing cut-off ranging from 10 to 40 µg Hb/g faeces using OC-Sensor (Eiken Chemical Co. Ltd, Tokyo, Japan), with only modest effects on detection rates of screen relevant neoplasia, classed as advanced adenoma or early stage colorectal cancer.

Further support for FIT as a screening tool comes from the recent interim report by Quintero *et al*. (2012) on a RCT being conducted in 57,404 subjects aged 50-69 years to compare biennial FIT at a cut-off of 15 µg Hb/g faeces using OC-Sensor with a strategy of offering one-time colonoscopy. The interim results show that one-time screening with FIT shows a similar detection rate for colorectal cancer as colonoscopy, with no significant difference in stage of tumours detected. Although more adenomas were identified with colonoscopy, uptake with significantly lower in this arm compared to that of the FIT group. The completion of the 10 year trial is awaited with much interest as to their full findings on the comparative effectiveness of the strategies for reducing colorectal cancer mortality.

1.3 Guaiac faecal occult blood tests v. Faecal Immunochemical Tests for haemoglobin

When selecting the most appropriate screening test, programme organisers must carefully consider its clinical performance in a screening setting. Four possible outcomes of a screening test result exist - true positive, false positive, false negative and true negative - and important indicators of the clinical performance of a screening test can be assessed from calculations involving the numbers of participants in each group, as outlined in Figure 1.1 in the context of colorectal cancer detection. Positive Predictive Value (PPV) in colorectal cancer screening is the proportion of participants undergoing colonoscopy as follow-up to a positive test result who actually have disease detected. Negative Predictive Value (NPV) is the proportion of participants with a negative test result who are, in fact, free from the disease in question e.g. correctly predicted as not having colorectal cancer. Sensitivity is a measure of the test's ability to identify the disease being screened for, e.g. the proportion of participants in the screened population who have colorectal cancer, who are identified via a positive screening test result. Conversely, test specificity is how well the test rules out those without disease. Other indicators of the clinical performance of a screening test include detection rate (number of true positives relative to the number of persons participating), and number needed to scope (NNS) to detect disease. In colorectal cancer screening (as in other average-risk population screening programmes), the vast majority of participants will have true negative test results. As a result, significant gains in sensitivity achieved by, e.g., lowering of the cut-off concentration, are associated with much smaller changes in specificity. These small reductions in specificity can, however, result in a substantial increase in those with false positive test results, with an

associated impact on costs and colonoscopy workload and this must be taken into account.

Figure 1.1 2x2 contingency table of possible colorectal screening outcomes with equations for calculation of indicators of test performance.

		Detection of colorectal cancer (CRC) at colonoscopy		
		CRC present	**CRC absent**	
Result of test for haemoglobin in the faeces	**Positive**	True positives (TP)	False positives (FP)	Positive Predictive Value: TP / (TP + FP)
	Negative	False negatives (FN)	True negatives (TN)	Negative Predictive Value: TN / (FN + TN)
		Sensitivity: TP / (TP + FN)	Specificity: TN / (FP + TN)	

Clinical performance of FIT in comparison to gFOBT has now been investigated in several studies, with RCT showing that FIT perform better in the detection of screen-relevant neoplasia. Van Rossum *et al*. (2008) compared the performance of a standard gFOBT to the OC-Sensor FIT by inviting 20,623 of the general population in The Netherlands, aged 50-75 years, randomly assigned to complete either three traditional two-sample gFOBT cards or a single quantitative FIT with a cut-off concentration of 20 µg Hb/g faeces. From results of colonoscopies performed on 103 gFOBT-positive participants and 270 FIT-positive participants, it was found that 2.5 times more cancers and advanced adenomas were detected with FIT than with gFOBT. However, the NNS to detect one cancer was comparable between the tests,

no significant difference was seen in the PPV for cancer and advanced adenomas, and specificities favoured gFOBT.

In another RCT, Hol *et al*. (2009) evaluated the performance of traditional gFOBT in comparison to OC-Sensor FIT at various cut-off concentrations between 10 and 40 µg Hb/g faeces in subjects aged 50-74 years in a screening-naïve population in The Netherlands. They concluded that FIT was a more effective screening tool than gFOBT at all seven cut-off concentrations examined owing to its superior performance with regard to both uptake and detection rate of colorectal cancer and higher-risk adenomas combined, termed advanced neoplasia. In keeping with the trial by van Rossum *et al*., PPV for advanced neoplasia were not significantly different between the two tests and specificity was significantly lower with FIT.

A later publication by Hol *et al*. (2010) incorporating the same dataset showed the results of the RCT when additionally evaluating flexible sigmoidoscopy as a screening modality. A randomly selected study population of 15,011 individuals were invited using a 1:1:1 ratio to participate in screening completing either gFOBT, FIT or flexible sigmoidoscopy. Colonoscopy was indicated in those with a positive faecal test, or, in the flexible sigmoidoscopy group, when advanced neoplasia was detected. Uptake was higher in the FIT group (61.5%) than in both the gFOBT group (49.5%) and the flexible sigmoidoscopy group (32.4%). The difference in uptake between the two faecal tests was thought to be attributable to the more demanding sampling procedure associated with the gFOBT. The detection rate for advanced neoplasia was significantly higher for FIT and flexible sigmoidoscopy than gFOBT. Flexible sigmoidoscopy gave a superior diagnostic yield per 100 invitees (2.4 [95% CI: 2.0 – 2.8]) than seen in both faecal test groups (1.5 [95% CI: 1.2 – 1.9] and 0.6 [95% CI: 0.4 – 0.8] for FIT and gFOBT, respectively). The authors, therefore, recommended

consideration of a dual-mode screening programme, involving both FIT and flexible sigmoidoscopy.

A more recent trial by Levi *et al*. (2011) randomised participants aged 50-75 years from an average-risk population in Israel to a FIT or gFOBT screening arm. From the 4,657 offered FIT and 7,880 a high-sensitivity guaiac FOBT (sFOBT), FIT had higher sensitivity for cancer detection at 100% compared with 61.5% for sFOBT, although specificity was again lower, at 85.9% compared with 96.4% with the sFOBT. The study also found FIT to provide increased detection rates for advanced adenoma with an odds ratio of 2.7 (95% CI: 1.6 – 4.5), rising to 3.2 (95% CI: 1.8 – 5.4) on an intention-to-screen basis. The authors stated that FIT seemed to meet the objectives of mass screening for colorectal cancer outlined by the World Health Organization of detecting 50 prevalent cases of colorectal cancer per 10,000 persons completing the test. (Wilson & Jungner, 1968)

In addition to the four RCT detailed above, three very recent comparison studies have been conducted of gFOBT and FIT performance in screening settings.

Brenner & Tao (2013) have published results of their head-to-head comparison of three quantitative FIT and a traditional gFOBT in 2,235 first-time participants aged 50-79 years in the German colonoscopy-based screening programme. Unlike other comparison studies, they adjusted FIT cut-off concentrations to generate an equal positivity rate across the tests. At this universal test positivity rate of 5%, all three FIT outperformed the gFOBT for all performance indicators examined. All sensitivities were around 2-3 times greater with the three FIT, with around twice as many neoplasms detected overall and three times as many advanced neoplasms than with

gFOBT. Specificity was high with gFOBT at 95.4% for advanced neoplasia, but was higher with the three FIT at 96.8%, 97.1% and 97.4% for each. PPV and Negative Predictive Values (NPV) were also significantly higher with FIT. This study seems to offer strong backing to the argument for replacing gFOBT with FIT. However, caution must be taken with interpretation of these results due to a flawed sampling technique with poorly preserved faecal samples, and cut-off concentrations that maximise comparability of the tests but may not be the most cost-effective or feasible to apply in full roll-out into population screening.

Another similar population-based comparison of FIT and gFOBT was carried out in France by Raginel *et al*. (2013), who invited 19,797 individuals aged 50-74 years to complete two different quantitative FIT and one traditional gFOBT. Results from 1,075 participants referred for colonoscopy showed that when the tests were compared at the same positivity rates (1.6%) to give a similar number of colonoscopies, the number needed to screen to detect one case of advanced neoplasia was 30% lower with the first FIT than with gFOBT, and the NNS were 3.3, 2.3 and 1.8, for the gFOBT and the two different FIT, respectively. Impressively, for the same number of positive test results, the second FIT detected almost double the number of cases of advanced neoplasia than did the gFOBT. An important observation is that variation exists between different FIT, with receiver operating characteristic (ROC) curve analysis showing the second FIT to display superior accuracy in the detection of advanced neoplasia compared with the first FIT. The second FIT is, however, more expensive at present, so again programme organisers have to give careful consideration to cost-effectiveness analysis.

Another recent French-based study by Hamza *et al*. (2013) demonstrated improved clinical performance with FIT compared with gFOBT in 23,231 average-risk screening invitees aged 50-74 years. At a cut-off of 17 µg Hb/g faeces using FOBGold (Sentinel Diagnostics, Milan, Italy), the detection rate of FIT for non-invasive cancer was six times that of gFOBT and four-times higher for advanced adenoma. At all cut-off concentrations examined (ranging from 17 up to 60 µg Hb/g faeces), PPV for advanced adenoma with were far higher than with gFOBT (ranging from 34.3 - 41.5% with FIT, 18.2% with gFOBT). These three recently published studies provide additional support to the case for replacing gFOBT with FIT in colorectal cancer screening.

Further studies have also compared the clinical characteristics of gFOBT with FIT in screening settings, with several of these using a sFOBT. Allison *et al*. (2007) compared a sFOBT with FIT in 5,841 average-risk participants aged over 50 years. FIT had significantly superior performance characteristics than the sFOBT with higher specificity and PPV both for colorectal cancer (96.9% v. 90.1% and 5.2 v. 1.5%, respectively) and advanced adenoma (97.3% v. 90.6% and 19.1 v. 8.9%, respectively), and a lower test positivity rate (2.1% v. 3.2%), although sensitivity for colorectal cancer and advanced adenomas was not statistically significant between the two tests. An evaluation by Levi *et al*. (2006) comparing a group of 162 subjects aged 50-75 years with a positive sFOBT result undergoing colonoscopy with 151 subjects due for colonoscopy additionally completing a FIT, found identical sensitivity for the detection of advanced neoplasia between FIT and sFOBT (both 75%), but with a statistically significant increase in specificity with FIT (94% v. 34%). Similarly, Rozen *et al*. (2009b) evaluated the performance of the qualitative FIT with a stated cut-off concentration of 50 ng/ml against a sFOBT in a population scheduled for colonoscopy either due to a positive test result from the sFOBT, increased colorectal cancer risk, or mild symptoms. From 330 participants completing both tests, similar sensitivity was found between the

two tests but with vastly improved specificity for FIT at 94.0% compared to 59.4% for sFOBT and 2.1 colonoscopies conducted to detect one neoplasm compared to 8.1 colonoscopies required with the sFOBT. In contrast, Smith *et al*. (2006) found that FIT had higher sensitivity for cancer and significant adenomas (36.6% v. 19.5%), but their indirect measurement of specificity slightly favoured sFOBT (97.5% v. 96.6%).

Several other studies comparing FIT with a traditional gFOBT rather than a sFOBT have been conducted, with a common finding being that sensitivity is better with FIT, but associated with lower specificity (Graser *et al.*, 2009; Guittet *et al.*, 2007; Park *et al.*, 2010; Parra-Blanco *et al.*, 2010; Wong *et al.*, 2012). However, in a 2007 review, Young & Cole (2007) did not deem the diminished specificity commonly seen with FIT to be unacceptable when balanced with the gain in sensitivity. Moreover, the nature of modern quantitative FIT allows modification of the cut-off concentration to potentially counteract this effect to some extent.

Further reviews of the available literature comparing the gFOBT and FIT exist. The 2010 meta-analysis conducted by Zhu *et al*. (2010) comparing gFOBT and FIT in screening and surveillance of advanced neoplasia concluded that FIT performed significantly better than gFOBT, with higher sensitivity and specificity in the diagnostic cohort studies reviewed. However, a systematic review by Burch *et al*. (2007) focussing on the diagnostic accuracy of faecal tests in colorectal cancer screening concluded that there was no clear indication of either FIT or gFOBT showing superiority. Although there was some evidence of FIT having higher sensitivity and specificity, this did not occur consistently and there were few direct comparisons available. Similarly, American guidelines (Levin *et al.*, 2008) state that there are no clear patterns of superiority in test performance between sFOBT and a variety of FIT, and that other factors such as cost-effectiveness and screening uptake should

therefore be considered by policy makers when selecting a test kit. Indeed, an evaluation of using quantitative FIT in two Scottish NHS Boards concluded that, despite clinical outcomes being similar to those observed with the two-tier reflex gFOBT followed by qualitative FIT algorithm in place, introduction of qualitative FIT was supported by various other advantages. These included improved uptake, good analytical reproducibility, removal of the possibility inter-observer variation and elimination of a fluctuating test positivity rate owing to batch-to-batch variation in reagents, a problem previously experienced in the programme. (Steele *et al.*, 2013)

Growing evidence demonstrates that FIT displays superiority over gFOBT specifically for adenoma detection, documented well in the review by Rabeneck *et al*. (2012) comparing the two types of faecal test. This observation is highlighted in the study conducted in France by Guittet *et al*. (2007) during the first year of a screening programme offered to 50-74 year olds using a standard gFOBT, with participants also asked to complete a Magstream FIT (Fujirebio Inc., Tokyo, Japan) with a relatively low cut-off concentration of 20 ng/ml (conversion factor for µg Hb/g faeces unavailable). 644 participants who had a positive test result from one of the two faecal tests had colonoscopy results available. It was observed that raising the cut-off concentration to 75 ng/ml would give the same test positivity rate with both tests (2.4%), with FIT having superior PPV for neoplasia than gFOBT, particularly for high-risk adenoma where the PPV was 49.2% compared with 27.7% with gFOBT.

Hundt *et al*. (2009) compared six different qualitative FIT kits and one gFOBT for detection of colorectal cancer precursor lesions in average risk participants in Germany undergoing colonoscopy. They found that the different tests had widely varying performance characteristics but FIT performed better for advanced adenoma detection with sensitivity and specificity ranging from 25% to 72% and 70% to 97%, respectively,

for the FIT, but just 9% and 96%, respectively, for gFOBT. The results from these studies suggest that, although overall comparison of performance characteristics between FIT and gFOBT is inconclusive, the real benefit of FIT in terms of clinical performance may lie in superior detection of precursor lesions – a key aim for an effective screening programme.

Further support for the replacement of gFOBT with FIT in screening may come from the 2011 report by Scholefield *et al*. (2012), detailing follow-up the participants of the RCT of biennial gFOBT conducted in Nottingham after 20 years. In the 152,850 individuals tracked, they found a reduction in colorectal cancer mortality in the intervention arm of 13% compared with the control arm. With an uptake of 57%, this figure rose to 18% when adjusted for actual screening participants. However, no significant reduction in colorectal cancer incidence was apparent despite 615 large precursor lesions being removed. It was suggested that a higher detection and removal rate would be required before an effect on incidence would be observed. With FIT having been shown to exhibit superior detection of adenomas, perhaps its incorporation into colorectal cancer screening programmes could have a greater impact on reducing colorectal cancer incidence. Indeed, a commentary on the study by Scholefield *et al*. went as far as to suggest that gFOBT is now obsolete in colorectal cancer screening and its replacement with FIT would not only enhance the observed reduction in colorectal cancer mortality but also contribute towards an impact on colorectal cancer incidence reduction through greater sensitivity for adenoma. (Young *et al.*, 2012)

It is important when selecting which screening test to implement that the constraints of the available resources are taken into account. An informative review by Young *et al*.(2015) considered the most appropriate test within four different scenarios: 1) limited

colonoscopy capacity; 2) expectation of maximum detection of neoplasia; 3) “adequate” colonoscopy capacity; and 4) aim of maximising screening uptake. The authors concluded that quantitative FIT is the test of choice for all four scenarios with the adjustable cut-off making it convenient for 1), 2) and 3), and superior for scenario 4). gFOBT was deemed only to be suitable for scenario 1), where there is a real need to constrain the test positivity rate.

1.4 Effects of adjusting the cut-off concentration with a Faecal Immunochemical Test for haemoglobin.

Any gain in sensitivity obtained by replacing gFOBT with FIT in a colorectal cancer screening programme can potentially be augmented by a range of further adjustments to screening strategy. However, improvement in sensitivity is only worthwhile if associated with an acceptable change in specificity to minimise false positive test results and maintain a referral rate that can be supported by the available colonoscopy resource. Scotland, like many other countries, has had to develop its colorectal cancer screening programme to operate within a relatively limited capacity for colonoscopy. A number of strategies can potentially be employed to minimise the burden placed on this resource by faecal testing. These include narrowing the age range, lengthening the screening interval, increasing the number of samples with referral only triggered by more than one positive test result, and limiting follow-up referrals in those with pathology detected. A further option afforded by quantitative FIT is setting the cut-off concentration to deliver a test positivity rate appropriate to the number of referrals that can be handled by the available colonoscopy resource.

Several studies have assessed the impact of modification of the cut-off faecal Hb concentration on various performance indicators associated with colorectal cancer screening. As would be expected, a general trend has been identified from the published data showing that as the cut-off concentration is raised, sensitivity for colorectal neoplasia detection decreases and specificity increases. (Grazzini *et al.*, 2009; Guittet *et al.*, 2009; Launoy *et al.*, 2005; Levi *et al.*, 2007; Nakama *et al.*, 2001; Rozen *et al.*, 2010; Wilschut *et al.*, 2011a) Similarly predictable effects when increasing the cut-off faecal Hb concentration, such as diminished detection rates, increasing PPV and decreasing NPV for cancer and adenomas, were additionally reported in further studies (Brenner *et al.*, 2010; Castiglione *et al.*, 2002; Kovarova *et al.*, 2012; Terhaar sive Droste *et al.*, 2011). To identify the cut-off concentration at which optimal clinical performance is achieved, the concentration at which there is an acceptable trade-off between these characteristics must be established. Numerous publications now exist documenting identification of this optimal concentration in various FIT screening settings.

Van Rossum *et al*. (2009) studied 428 average risk participants aged 50-70 years old undergoing colonoscopy following a faecal Hb concentration over 10 µg Hb/g faeces. Performance characteristics were also calculated at 15 and 20 µg Hb/g faeces and, as expected, detection rates and NNS to detect one colorectal cancer or advanced adenoma both fell as the cut-off concentration rose. From these results, the authors advocated the use of a cut-off faecal Hb concentration of 15 µg Hb/g faeces, given sufficient colonoscopy capacity, and recommend increasing up to 40 µg Hb/g faeces where this is not the case. A rise in the cut-off concentration from 10 to 40 µg Hb/g faeces saw a 50% reduction in the number of colonoscopies, while the detection rate for advanced neoplasia fell from 3.1% to 1.8% and the NNS to detect one case of advanced neoplasia also fell from 2.3 to 1.8. The reduced detection rate for advanced

neoplasia when the cut-off concentration was raised from 20 to 40 µg Hb/g faeces was found to almost entirely relate to missed adenomas, with the detection rate for colorectal cancer unaffected. With progression of the adenoma-to-cancer pathway in the colon being a gradual process, this fall in adenoma detection rate may be acceptable in view of the potential for eventual detection of such lesions in subsequent screening rounds while they are still at an early stage of malignancy.

The randomised comparison of FIT and gFOBT performance carried out by Hol *et al*. (2009) also examined the effect of varying the cut-off faecal Hb concentration in screening. Although stating that a full cost-benefit analysis is essential for accurate determination of the ideal cut-off concentration at which the benefits associated with screening, i.e., early detection of colorectal cancer, outweigh the harms, such as costs, anxiety and complications associated with undergoing colonoscopy, Hol *et al*. considered that the ratio between detection rate and NNS acts as a good indicator of this. Their assessment of this balance gave findings in agreement those of van Rossum *et al*. in that a cut-off faecal Hb concentration of 15 µg Hb/g faeces was deemed to give an acceptable trade-off. Interestingly, they found that a considerably larger decrease in test positivity occurred when raising the cut-off faecal Hb concentration from 10 to 15 µg Hb/g faeces than that occurring with any further similar increase, with the trade-off between detection rate and NNS being less favourable when further raising the cut-off faecal Hb concentration to 20 µg Hb/g faeces.

Detailed cost-effectiveness analysis, such as that conducted by Chen *et al*. (2007), is of paramount importance in selecting a cut-off faecal Hb concentration that can be deemed optimal for all relevant parties. The study, conducted in Taiwan, invited 22,672 participants aged over 50 years to complete a FIT with a cut-off faecal Hb concentration of 20 µg Hb/g faeces; interval cancer data were collected on those with a

faecal Hb concentration below this concentration. An optimum cut-off concentration in terms of test performance was identified at 20 µg Hb/g faeces using ROC curve analysis with an area under the curve of 0.87 (95% CI: 0.81 - 0.93), at which sensitivity was 81.5% and the false positive rate 5.7%. Further to this, economic appraisal determined that the optimum number of life-years gained (LYG) and money saved occurred using a concentration equivalent to 22 µg Hb/g faeces. It was demonstrated that, as the cut-off faecal Hb concentration rose above 20 µg Hb/g faeces, the average number of LYG fell and average discounted costs started to rise. Although also related to the costs associated with referrals from false positive test results, this effect was found to be more attributable to the consequences of false negative test results, with cancers missed at screening associated with more expensive treatments when they later presented in a more aggressive form. Therefore, although using a high cut-off faecal Hb concentration will lower the costs associated with demand on the colonoscopy resource, particularly from false positive test results, greater adverse consequences may arise with the indirect costs associated with failure to detect colorectal cancer at an early stage.

Methods for adapting the screening programme to limit constraints on colonoscopy resource were further investigated by Wilschut *et al*. (2011a) using a series of simulated models. They assessed the effects of various screening strategies on the number of colonoscopies required, costs incurred and health outcomes. The adaptations simulated included narrowing the age range of invitees, reducing the number of screening interventions, employing a more specific test, and adjusting the cut-off faecal Hb concentration for a positive test result from 10 up to 40 µg Hb/g faeces. The best strategy at all cost levels, assuming unlimited colonoscopy capacity, was annual FIT at a cut-off faecal Hb concentration of 10 µg Hb/g faeces offered to 45-80 year olds. However, when operating within limited capacity for colonoscopy, the

authors state that the first step is to raise the cut-off faecal Hb concentration used to 40 µg Hb/g faeces. To further reduce the burden on colonoscopy resource, limiting the age range to 50-75 year olds, then finally lengthening the screening interval is recommended, according to the results of the simulated models. The impact of colonoscopy capacity on screening effectiveness was demonstrated by the finding that doubling this resource can increase LYG by up to 100%. It was pointed out that using an elevated cut-off faecal Hb concentration would generate a higher-risk group of participants with a positive test result, for whom the potential for the intended health benefit of colorectal cancer screening was greater. Indeed, it has become increasingly well documented that faecal Hb concentration relates to disease severity (Ciatto *et al.*, 2007; Hol *et al.*, 2009; Launoy *et al.*, 2005; Levi *et al.*, 2007; Rozen *et al.*, 2009a).

Further analysis of the effects of raising the cut-off faecal Hb concentration to control the number of screening colonoscopies performed has been conducted by Terhaar sive Droste *et al*. (2011) With participants aged over 40 years completing a single FIT, they assessed the impact of raising the cut-off faecal Hb concentration at increments between 10 and 40 µg Hb/g faeces on sensitivity and specificity for detection of screen-relevant neoplasia, namely advanced adenoma and early-stage colorectal cancer. Test positivity rates ranged from 16.5% to 10.2% as the cut-off faecal Hb concentration was raised from 10 to 40 µg Hb/g faeces, representing a 42% reduction in the number of colonoscopies required. The associated 6% drop in detection rates of early stage colorectal cancer was deemed acceptable. Detection of advanced adenoma was diminished to a greater extent when raising the cut-off faecal Hb concentration, although, consistent with van Rossum *et al*., (2009) this was not viewed as too great a concern due to the potential for missed adenoma to be detected at subsequent screening rounds, with the long phase of adenoma to colorectal cancer disease progression meaning that the missed lesions would likely either still be presenting in a

subsequent round as an adenoma, or as a treatable early-stage cancer where colorectal cancer death remains preventable.

It is evident that optimal cut-off faecal Hb concentration identified from the numerous analyses conducted will not be directly applicable across different geographic populations. Each country looking to incorporate quantitative FIT into their colorectal cancer screening programme will need to carry out their own evaluations relevant to the specific screening population to be invited prior to selection of their cut-off faecal Hb concentration. This should take into account numerous factors including disease prevalence, resource availability and cost-effectiveness. Raising the cut-off faecal Hb concentration should be considered when colonoscopy capacity is limited, at the expense of detection rates which will suffer a gradual decline as the cut-off faecal Hb concentration is increased.

1.5 Length of the screening interval

Colorectal cancer prevention through screening is optimised by repeated invitations. Intermittent bleeding is associated with adenomas, particularly at an advanced stage (Chen *et al.*, 2007), meaning that many participants with a negative faecal test result may harbour advanced adenomas not bleeding consistently enough to give a positive test result, but that are susceptible to malignant transformation over time. Repeated testing allows further opportunity for relatively early detection and removal of such lesions. The length of time between each screening round must be decided by programme organisers based on resource availability and recommendations for optimal use of the screening modality made in the available literature.

Van Roon *et al*. (2013) randomly invited 7,501 screening-naïve Dutch participants aged 50-74 years to complete two rounds of single-sample FIT screening with an interval length of either one, two, or three years. As would be expected in a previously unscreened population, the test positivity rate and advanced neoplasia detection rate was lower in the second round with all three interval lengths. In addition, detection rate at the second round was not dependent on the interval length. The authors suggested that this may mean that the screening interval can be tailored to local resources. For example, when resources are limited, a longer interval can be used without significantly affecting test positivity and advanced neoplasia detection rates. Interval cancers arising in the study population were also analysed. Although only three interval cancer cases were identified, two were in the group invited triennially, accounting for 20.0% of colorectal cancer in that group. The remaining interval cancer case arose in the biennially invited group, representing 9.1% of cancers in that group.

Two studies have been published using microsimulation models to determine the optimal interval between rounds in colorectal cancer screening. Modelling by Zauber *et al*. (2008) to measure LYG compared with no screening demonstrated that successive annual rounds of sFOBT or FIT screening has preventative effects similar to those seen with colonoscopy offered once every 10 years, and to 5-yearly sigmoidoscopy. In general, a longer screening interval was associated with fewer LYG. Wilschut *et al*. (2011b) examined cost-effectiveness of quantitative FIT using various cut-off faecal Hb concentration and screening intervals of 1, 1.5, 2 and 3 years in their models. They found that biennial FIT was cost-effective in terms of LYG. An interesting point made was that shorter intervals compensate for suboptimal uptake of screening, where those not participating in one round soon have another opportunity. However, shorter intervals were not necessarily found to be optimal for regular participants. Indeed, van Roon *et al*. (2013) observed enhanced test uptake in the second round in those invited

biennially and triennially compared with those participating annually, with statistical significance reached with triennial compared with annual invitation. Considerable evidence showing quantitative FIT to be associated with improved uptake compared with gFOBT may mean that a shift towards longer screening intervals could become the more cost-effective route.

Further advantage gained from introduction of quantitative FIT may be that there is scope for participants with a negative test result to be identified as being at high-risk due to a faecal Hb concentration close to the cut-off faecal Hb concentration perhaps being invited for their subsequent screening round at a shorter interval than those who are deemed low-risk. This hypothesis was made by Chen *et al.* (2011) in conclusion to their important investigations into faecal Hb concentration as a predictor of colorectal neoplasia that will be discussed in more detail later in this Chapter, and is an idea that represents an interesting new area for research into more efficient use of quantitative FIT.

1.6 Number of samples

An additional measure to improve detection rates whilst reducing false positive test results could be to increase the number of faecal tests to be completed per round of colorectal cancer screening. The reasoning behind the use of multiple samples again comes from the knowledge that some lesions are associated with intermittent bleeding, affecting the sensitivity and specificity of screening with FIT. Numerous studies have been conducted investigating how many samples are required to optimise measures of diagnostic accuracy, whilst maintaining cost-effectiveness and not adversely influencing participation rates.

A study by Goede *et al*. (2013) using a microsimulation model based on data from an RCT compared cost-effectiveness of one- v. two-sample FIT testing in colorectal cancer screening. Two-sample testing provided an additional 7.3 - 12.4 LYG compared with one-sample testing, with the accompanying extra costs deemed acceptable. However, it was also found that a similar or greater number of LYG with lower associated costs can be achieved by using one FIT and intensifying screening by means of widening the age range or shortening the screening interval and therefore recommended such strategies ahead of increasing the number of samples to achieve cost-effectiveness.

Oort *et al*. (2011) conducted a prospective cohort study with 1,096 subjects scheduled for colonoscopy completing two FIT. Clinical sensitivities and specificities were compared for when participants with an overall positive screening test result were identified by one of three different pathways: either one of the two test results positive, both test results positive, or the mean faecal Hb concentration of the two being above the cut-off used. At any of the cut-off faecal Hb concentration examined, the lowest sensitivity for screen-relevant neoplasia (early stage colorectal cancer or advanced adenoma) was in the group with both test results positive (range of 35 - 44%). Sensitivity was best when test positivity was considered with one out of two tests being above the concentration (range of 42 - 54%). ROC curves of double FIT sampling were similar to those calculated from single-sampling data and neither of the double-sampling strategies displayed a superior combination of sensitivity and specificity over single-sample FIT.

Van Roon *et al*. (2011) conducted a population-based trial in which 5,007 participants were invited to complete one FIT and 3,197 to collect faecal samples on two consecutive days. No difference in participation rate was seen between the two groups, at 61.5% and 61.3% respectively. Participants were referred for colonoscopy when at least one positive test result was obtained and test positivity and detection rates for advanced neoplasia in the single-sample group were compared with those calculated for three positivity strategies in the two-sample group, analogously to Oort *et al*. (one of two test results positive; both test results positive; mean of two test results above cut-off faecal Hb concentration). Using a cut-off faecal Hb concentration of 10 µg Hb/g faeces, two-sample FIT screening using a least one positive test result as the referral criteria provided a higher detection rate for advanced neoplasia than single-sample FIT screening (4.1% v. 3.1%). However, this was at the expense of higher test positivity rates (12.8% v. 8.1%), and therefore greater demand on colonoscopy resource. It was recommended that development of efficient screening strategies that can be adapted according to colonoscopy capacity rather than varying the cut-off faecal Hb concentration with single-sampling, pointing to their results showing that with test positivity rates up to 3.2%, two-sample FIT at a cut-off concentration of 20 µg Hb/g faeces, when demanding both test results to be positive for referral, provided the most-efficient strategy.

Similarly, Guittet *et al*. (2009) investigated the performance of FIT according to the number of samples whilst varying the cut-off faecal Hb concentration used. Single-sample screening displayed sensitivities and specificities comparable to those of the strategy requiring one positive test result from two samples to warrant colonoscopy. Taking the mean of the faecal Hb concentration of the two samples to determine a positive test result brought about improved performance at any test positivity rate generated. In contrast to the later study by van Roon *et al*, (2011) it was suggested

that the use of one positive test result from two tests should be replaced by single-sampling, or for even better clinical outcomes, a strategy using the mean faecal Hb concentration of two samples providing uptake remained at similar level to that seen with single-sampling.

The study by Wong *et al*. (2012) comparing performance of gFOBT and FIT also included analysis of single- against two-day FIT sampling. A trend towards superior sensitivity for colorectal cancer and adenoma was seen when one out of two samples having positive test results warranted referral compared with single-sampling and when two positive test results were demanded; however, the difference was not significant. False positive test result rates, however, were significantly higher when using one positive test result in two samples compared with the other two strategies. Single-sampling was deemed to be an acceptable option for screening with the aim of raising participation rates.

Further work examining the effect of the number of FIT samples completed on clinical performance comes from the analysis of Rozen *et al*. (2009a) of adenoma detection using FIT. 1121 participants scheduled for colonoscopy completed three consecutive daily FIT. Diagnostic power was calculated by comparing colonoscopy findings with the test result when using the first, the higher of the first two and the highest of the three faecal Hb concentration measurements. Sensitivity and specificity were assessed at various cut-off faecal Hb concentration between 10 and 30 μg Hb/g faeces, and at the higher end of this range, sensitivity increased and specificity decreased as the number of samples collected increased. These findings again demonstrate how, as well as by adjusting the cut-off faecal Hb concentration with quantitative FIT to suit colonoscopy capacity, programme organisers can modify the number of samples tested to give the best performing strategy. Detailed analysis of

cost-effectiveness will be required prior to any such decision to determine whether the extra costs associated with processing additional samples are justified by the benefits gained from the potentially more sensitive screening strategy.

Levi *et al*. (2007) investigated the performance of quantitative FIT in a population at increased risk of colorectal cancer. 1,000 participants collected three samples and faecal Hb concentration were compared with colonoscopy results. They found that at a cut-off faecal Hb concentration of 15 µg Hb/g faeces, detection of colorectal cancer when taking the highest faecal Hb concentration of three was the same as when using the higher of the first two faecal Hb concentration (area under the curve 0.96, [95% CI: 0.94 - 0.98] and 0.96, [95% CI: 0.93 - 0.98], respectively), but taking the first faecal Hb concentration only diminished diagnostic performance (area under the curve 0.87 [95% CI: 0.78 - 0.97]). Similar findings, but to a lesser extent, were seen when also performing a ROC curve analysis for advanced neoplasia. As such, it was stated that use of the highest of the faecal Hb concentration found in two or three samples is superior to single-sampling in terms of diagnostic performance.

The European Guidelines for Quality Assurance in Colorectal Cancer Screening and Diagnosis (Halloran *et al.*, 2012) included a review of existing studies examining the effect the number of faecal samples used in colorectal cancer screening. In summary, it was recommended that adoption of screening strategies involving collection of more than one sample with specific test positivity criteria should be considered to give an appropriate referral rate in terms of cost-effectiveness, clinical performance and logistical feasibility.

From the studies including analysis in this facet of the use of FIT, it is evident that modifying the number of samples collected per participant is a potential adaptation to programme strategy that warrants consideration when seeking to improve screening effectiveness with faecal tests. It is, however, a complicated matter, to be guided by various associated factors including participation rates, available resources and cost-effectiveness.

1.7 Age and gender differences in colorectal cancer screening

Another important area identified for potential improvement in the efficiency of colorectal cancer screening is to take into account age and gender differences in screening detection rates. The imbalance between different age groups and genders, in colorectal cancer screening in terms of test positivity rates and disease prevalence is well established, having been identified in several studies.

Steele *et al*. (2010) investigated the effect of age and gender on key performance indicators generated in the Scottish Bowel Screening Programme using biennial gFOBT and reported significant increases in test positivity rates, colorectal cancer detection rates, and PPV for colorectal cancer with age, and also in men compared with women for all ages.

Furthermore, prevalence of colorectal neoplasia among individuals with a negative gFOBT result was studied by Rex *et al*., (1993) who showed, using multivariate analysis, that increasing age and male sex were both strong indicators of colorectal neoplasia. Their data showed a particularly substantial prevalence among elderly men.

Regula *et al*. (2006) used multivariate logistic regression to identify associations between advanced neoplasia detection and participant characteristics in a colonoscopy-based screening programme, and confirmed the findings on a validation data set. When adjusted for age and family history, male sex was found to be associated with advanced neoplasia, with an adjusted odds ratio of 1.73 (95% CI: 1.52 - 1.98). An odds ratio of 2.91 (95% CI: 2.21 - 3.83) was calculated for those aged 60-66 years compared with those aged 40-49 years. At all ages, the NNS to find one case of advanced neoplasia was significantly lower in men than in women. To address this discrepancy, the authors suggest the strategy of only offering screening to population groups with a NNS below a pre-determined threshold, but stress that this needs to be backed up by cost-effectiveness analysis.

In a study conducted to assess how neoplasia detected in the distal colon predicts the risk of neoplasia found proximally, Imperiale *et al*. (2000) found older age and male sex to be associated with higher rates of advanced neoplasia in the proximal colon, with relative risk reported as 1.3 for every five years of age and 3.3 for male gender.

A review of the literature detailing gender as a risk factor conducted by Nguyen *et al*. (2009) calculated a pooled risk ratio for advanced neoplasia of 1.83 (95% CI: 1.69 - 1.97) for men compared with women, rising to 2.02 (95% CI: 1.53 - 2.66) for colorectal cancer. Again, the NNS to detect advanced neoplasia was lower in men than in women, in all age groups studied. Possible reasons listed for the gender imbalance included smoking prevalence and alcohol intake, use of hormone replacement therapy (HRT), Body Mass Index (BMI) and genetic factors. Diet and use of medications such

as aspirin and non-steroidal anti-inflammatory drugs are also potential contributors to the observed gender imbalances.

Kolligs *et al*. (2011) investigated the risk of advanced neoplasia according to age and gender, by analysing results of 625,918 colonoscopies. To determine whether screening should start at an earlier age according to gender, the analysis included colonoscopies performed in those within the age range 18-79 years. Advanced neoplasia was detected in 8.6% of men, compared with 4.6% of women. Logistic regression modelling was used to determine age- and gender-specific risk of advanced neoplasia. Men were found to be at higher risk of advanced neoplasia than women at any age, with an overall odds ratio of 1.93 (95% CI: 1.89 - 1.97). Comparable numbers of colonoscopies needed to detect advanced adenoma were reached up to 20 years earlier in men than in women. The authors suggest starting screening earlier in men to increase detection rates relevant to their increased risk. However, with Kolligs *et al*. (2011) also stating that lifetime risk of colorectal cancer appears fairly similar for both men and women, the findings lead to speculation that some mechanism exists whereby development of advanced neoplasia is delayed in women. Possible driving factors include protective hormonal effects in pre-menopausal women, and then continuation of this through HRT use during the menopause, as well as preferential lifestyle choices adopted by women such as lower smoking rates and alcohol intake.

Evidence of a potentially delayed process along the pathway to malignancy comes from a study by Brenner *et al*. (2007b) investigating the progression of advanced adenoma to colorectal cancer by age and gender. They found similar rates of transition to colorectal cancer between men and women, with a strong gradient existing for age in both genders. However, they identified a greater increase in advanced adenoma prevalence when comparing the 50-59 years to the 80 years and over age

groups in women, (3.4% to 7.3%), than in men (6.2% to 9.5%). In addition, an even stronger relationship existed between increasing age and colorectal cancer incidence, with an increase greater than five-fold observed in women between the two age categories, whereas men had a more than four-fold increase. Similarly, projected annual and ten-year percentages of advanced adenoma progression to colorectal cancer were estimated to be higher in women than men at later ages, particularly at 70-80 years. This may add strength to the hypothesis that the development of neoplastic lesions in women is delayed by hormonal factors and lifestyle choices.

Further backing to this idea comes from work conducted by Schoenfeld *et al*. (2005) It was demonstrated that for those aged 60-69 years, a significantly higher prevalence of advanced neoplasia existed in men than in women, and a trend towards this was observed in those aged 50-59 years, but not in those aged over 70 years. As with the findings of Brenner *et al*. (2007b), this has importance in helping to explain the age and gender differences in disease prevalence. The narrowing of the gender gap in prevalence of advanced neoplasia with increasing age supports the theory of women experiencing protective hormonal effects, which disappear with age, perhaps along with the benefits of women making more favourable lifestyle choices than those adopted by men. Another interesting discovery from the data of Schoenfeld *et al*. was that a lower diagnostic yield of flexible sigmoidoscopy was evident among women than in men, pointing to a shift to right-sided neoplasia in women. A particular concern pointed out was that 70% of advanced neoplasia in women aged 50-59 years would be missed using flexible sigmoidoscopy alone as a screening tool. This ties in with findings from studies on interval cancer using gFOBT in colorectal cancer screening showing a disproportionate number of cases occurring in women with proximal lesions (Gill *et al.*, 2012; Steele *et al.*, 2012). In the case of gFOBT screening, a commonly stated possible reason for this finding is a slower colonic transit rate in women

compared with men, allowing a longer time scale for faecal Hb degradation and therefore a negative test result. With right-sided lesions, this effect would be accentuated by the further distance that blood will have to travel through the colon in these cases before defaecation.

Rozen *et al*. (2012) further highlight the influence of age and gender on screening results in their follow-up analysis of 1,630 who had undergone colonoscopy and had completed three FIT with a cut-off faecal Hb concentration set at 10 µg Hb/g faeces. Linkage of data from the Israel National Cancer Registry with mean follow-up of patients over 51.5 months showed colorectal cancer and advanced adenoma to be significantly more common in men than women. Using a reference category of patients aged under 50 years, it was calculated that men had an increased relative risk (4.639) of developing advanced neoplasia between the ages of 51-73 years, but this was not observed among women. However, at 74 years or older, the relative risk of colorectal cancer or advanced adenoma increased significantly in both genders. Although it would be useful to see changes in relative risk in narrower age groups than reported in this study, the finding that risk of advanced neoplasia increased at a younger age in men than it did in women once more indicates that some delay in disease progression may exist in women.

A further study by Brenner *et al*. (2007a) investigated gender differences in colorectal cancer incidence in respect of the implications they may have for recommendations concerning age for initiation of screening. Following their analysis of data from the United States, they state that as the potential benefits of screening are heavily influenced by colorectal cancer incidence and mortality rates at various ages, the optimal screening start age would be around five years earlier for men than for women when aiming to improve cost-effectiveness. However, this would be based on the

assumption that screening is equally effective in both genders. The disproportionately high number of interval cancers in women shown with gFOBT, and the potentially high false negative rate of flexible sigmoidoscopy in younger women suggest this may not be the case.

In a more recent paper, Brenner *et al*. (2010) documented the prevalence of advanced neoplasia in 1,157 men and 1,167 women in the German screening programme at range of cut-off faecal Hb concentration for quantitative FIT, as well as six qualitative FIT and a gFOBT. They found a substantially higher prevalence in men than in women (13.5% v. 7.5%) and observed that at any cut-off faecal Hb concentration, and with all other tests examined, sensitivity and PPV were significantly higher and specificity and NPV were significantly lower among men than women. These findings were particularly striking for the gFOBT, perhaps suggesting that the advantages offered by FIT may go some way to reducing the gap in test performance between men and women. Indeed, it was established that gFOBT performed no better in women than would random selection for referral for colonoscopy and was therefore deemed unable to differentiate between those who do and do not have disease. A possible reason for gender differences in test performance could be that men have higher average faecal Hb concentration than women, and therefore more positive screening test results arise in men.

Evidence for this comes from McDonald *et al*., (2012) who studied the distribution of faecal Hb concentration by age and gender in 38,720 screening participants completing one quantitative FIT. At any single faecal Hb concentration, test positivity was higher in men than women as well as in older participants than in those younger. An observational study documented variation in distributions of faecal Hb concentration across geography when comparing data from Scotland, Taiwan and Italy but, in all

three datasets, faecal Hb concentration was higher in men than women and in older than younger subjects. (Fraser *et al.*, 2014) This work was supplemented by the same trends being found in the prevalence screening round of the Barcelona Colorectal Cancer Screening Program (Fraser & Auge, 2014) where it was stated that test positivity rate should be viewed as a surrogate marker for faecal Hb concentration. Further confirmation of this idea has come from recent data presented in an important study from Australia. (Symonds *et al.*, 2015b) Correspondence generated in light of these findings recommended that when selecting an appropriate cut-off faecal Hb concentration for colorectal cancer screening using quantitative FIT, programme organisers should perform pilot studies to examine faecal Hb concentration by age and gender, with characteristics of the invited population assessed regularly with evolution of the programme to avoid the problems that have been seen in some countries where colonoscopy services have been overwhelmed by the demands of significantly higher than expected test positivity rates. (Fraser, 2015) A letter submitted as an addendum to the published results of Symonds *et al.* (2015a) then stated that men were found to have statistically significantly higher median faecal Hb concentration than women among those who had no colorectal disease at colonoscopy, indicating that the fact that there is a greater prevalence of colorectal neoplasia in men than women is not the sole reason for the disparity in baseline faecal Hb concentration between the genders. Test sensitivity for advanced neoplasia at a faecal Hb concentration cut-off of 10 µg Hb/g faeces was 50.4% in men compared with 42.7% in women, and a faecal Hb concentration cut-off of 5.4 µg Hb/g faeces would have been required to detect the same proportion of advanced neoplasia in women as in men. The authors stated that these findings supported the argument that gender differences in test sensitivity may lead to women having a greater proportion of interval cancers and missed adenomas than men in population screening and that further work is needed to devise an optimal strategy providing equity of screening within the constraints of the colonoscopy resource.

Additionally, faecal Hb concentration has been shown to increase according to lesion size in those with colorectal neoplasia detected (Ciatto *et al.*, 2007). This is relevant to the findings of Brenner *et al*., (2010) who reported a higher proportion of colorectal cancer and large adenoma among men with neoplasia than women with neoplasia, meaning that the neoplasia in men was more likely to be associated with higher faecal Hb concentration and therefore higher test sensitivity. However, when advanced neoplasia was stratified by size, sensitivity, albeit somewhat reduced, was still substantially higher in men than women, suggesting that differences in neoplastic pathology was only partly the reason for the disparity in sensitivity between genders.

A review of gender-specific and site-specific differences in colorectal cancer screening by Massat *et al*. (2013) stated that biennial gFOBT screening was equally effective for men and women, but gender differences in test performance between gFOBT and FIT had not been investigated with enough power to draw conclusions. Thus, a strong recommendation was made that researchers publish results by gender whenever possible.

From the evidence published thus far on age and gender differences in colorectal cancer prevalence and detection, it is apparent that this is a facet for programme organisers to address when seeking to improve screening effectiveness. Although there is clearly a greater prevalence of advanced neoplasia amongst men than women, some screening modalities are associated with a higher number of false negative test results in women. It has been shown that gFOBT may disadvantage women, particularly those with right-sided lesions. Therefore, careful consideration must be made with regard to adjustment of screening programmes such as use of a gender-

specific age of screening commencement. A very recent study has emerged from The Netherlands documenting gender differences observed in FIT screening using a faecal Hb concentration cut-off of 10 µg Hb/g faeces. (Kapidzic *et al.*, 2015) Men were found to have a significantly higher test positivity rate, advanced neoplasia detection rate and false positive rate than seen in women. The authors recommend use of the same faecal Hb concentration cut-off in men and women based on no significant differences in PPV for advanced neoplasia by gender. However, further research is crucial to understanding the mechanisms driving differing average faecal Hb concentration between genders, a possible shift to right-sided lesions in women and the apparent delay in dysplastic progression in women which may be alluded to from a number of studies discussed previously. With Brenner *et al*. (2010) showing that replacing gFOBT with FIT may go some way to reducing the gender gap in test performance, using quantitative FIT with age- and gender-specific cut-off faecal Hb concentration for a positive test result also seems worth investigating with the aim of optimising screening efficiency and reducing interval cancer. Before such tailoring of screening programmes using quantitative FIT, it is crucial firstly to understand the importance of faecal Hb concentration as a predictor of colorectal disease. Some studies have been conducted to investigate what correlation exists between faecal Hb concentration and colonoscopy findings.

1.8 Significance of faecal haemoglobin concentration in colorectal disease, and risk scoring

Levi *et al*. (2007) investigated faecal Hb concentration in 1,000 consecutive subjects scheduled for colonoscopy. They found that those with advanced neoplasia had significantly higher faecal Hb concentration that those with other diseases or no pathology detected. Although no faecal Hb concentration perfectly distinguished those

with advanced neoplasia from others, most non-advanced adenomas were associated with low faecal Hb concentration (< 15 µg Hb/g faeces) meaning false positive test results arising from low-risk adenoma in screening using FIT should be limited. In addition, differences in faecal Hb concentration were observed between adenomas with differing histological characteristics. Patients with adenomas displaying villous or serrated components, or high-grade dysplasia - all features associated with greater malignant potential (Lieberman *et al.*, 2012) - had higher faecal Hb concentration than those with lower risk adenomas, or no neoplasia. No variation in faecal Hb concentration was observed between advanced adenoma found in the proximal region of the colon and those found more distally. The overriding finding was that lesion size was highly related to faecal Hb concentration, with small proximal cancer and small advanced adenoma both being associated with low faecal Hb concentration. This seems to suggest the detailed histology of the lesion is not so much responsible for causing bleeding as is the more simple explanation that the larger the lesion, the more susceptible its associated vasculature is to interference from the mechanical effects of faeces passing through the colon.

Rozen *et al*. (2009a) also correlated faecal Hb concentration with adenoma characteristics, collecting three daily samples for FIT from 1221 individuals scheduled for colonoscopy. Those with adenoma had elevated faecal Hb concentration, which increased with advanced histology, size, pedunculated shape and multiplicity. As with the findings of Levi *et al*., (2007) adenomas with villous or serrated features, or those displaying high-grade dysplasia had higher faecal Hb concentration than those without these features. Again, size was cited as an important factor in the degree of bleeding associated with a lesion, with no difference in faecal Hb concentration between those with small adenomas and those with no neoplasia. Pedunculated adenomas were larger than sessile adenomas, and it can be deduced that this, in combination with their

more protruding shape, is the reason for their elevated faecal Hb concentration. Advanced adenoma had a larger mean size than non-advanced adenoma. No significant difference was found in faecal Hb concentration or size of adenomas between proximal and distal sites. Again, most non-advanced adenoma were undetected by FIT at the cut-off concentration used of 15 µg Hb/g faeces; advantageous in avoiding unnecessary follow-up.

Ciatto *et al*. (2007) discovered similar results from their multivariate analysis showing age, lesion size and left-sidedness to show independent association with increasing faecal Hb concentration in adenomas. A trend of increasing faecal Hb concentration according to colonoscopy findings was evident, from benign outcomes through a spectrum of disease severity up to colorectal cancer. Advanced adenomas (classified as those > 9 mm in diameter, > 20% villous or tubulovillous histological pattern, or displaying high-grade dysplasia) had significantly greater faecal Hb concentration than non-advanced adenoma. High-grade dysplasia was associated with larger adenoma, with increasing size also associated with villous histology in adenoma. Adenoma found in the left colon were larger than right-sided adenoma. The authors suggest that the association between elevated faecal Hb concentration and left-sided adenoma may be attributable to a greater effect of the mechanical action of faeces in this section of the colon since the faeces is more formed. Multivariate analysis further consolidated size as key to a lesion's propensity to bleed with this being the factor to show the strongest association with faecal Hb concentration.

Further work demonstrating a continuum of increasing faecal Hb concentration with increasing severity of colorectal disease comes from Kovarova *et al*., (2012) who showed that, in 682 participants completing two FIT prior to complete colonoscopy, median faecal Hb concentration increased from normal, through adenoma, to

advanced adenoma, to colorectal cancer. However, as with the other studies discussed, much overlap was apparent.

The findings of these studies confirm the importance of faecal Hb concentration as an indicator of colorectal neoplasia. In addition, an important recent study provides a significant message that not only is elevated faecal Hb concentration related to increased risk of colorectal cancer mortality, but also a similar, although less marked, increased risk of all-cause mortality. (Chen *et al.*, 2013) Those with faecal Hb concentration above 90 µg Hb/g faeces had an adjusted hazard ratio for all-cause mortality of 1.67 (95% CI: 1.54 – 2.07) as compared to the baseline group with faecal Hb concentration 1.0 - 3.9 µg Hb/g faeces. This work emphasises the importance of faecal Hb concentration as a predictor of disease and the contribution it can make to detection of colorectal cancer through screening. The technological advancement in faecal testing facilitated by quantitative FIT allows potential for better use to be made of faecal Hb concentration measurements obtained from screening participants. Risk scoring systems that include faecal Hb concentration as a variable may be a step towards such use of FIT to improve screening efficiency.

An increasing number of studies focussing on the use of risk scoring models in colorectal cancer screening programmes are emerging. (Chen *et al.*, 2014; Driver *et al.*, 2007; Kaminski *et al.*, 2014; Wang *et al.*, 2014; Wong *et al.*, 2014; Yeoh *et al.*, 2011) Common risk factors included in such models include gender, age, BMI, smoking status etc., but only very few have incorporated faecal Hb concentration. (Aniwan *et al.*, 2015; Stegeman *et al.*, 2014) A very recent publication from Thailand (Aniwan *et al.*, 2015) combined the Asia-Pacific Colorectal Screening score (Yeoh *et al.*, 2011) incorporating age, gender, family history of colorectal cancer and smoking status, with the result of a qualitative FIT set at 50 ng Hb/ml buffer (equivalent to 10 µg Hb/g

faeces), in 948 asymptomatic participants aged 50-75 years. They found that those who were both at high risk according to their Asia-Pacific Colorectal Screening score, and had a positive test result, had a 6.15-fold higher detection rate for advanced neoplasia compared with those in the other three groups (high risk + negative test result, moderate risk + positive test result, moderate risk + negative test result). Of significant interest here is a measure of the improvement that FIT brings to the performance of the scoring system. The prevalence of advanced neoplasia in participants calculated as being at high risk using the score alone was 19.8%. This rose to 36.9% in those with a high risk score and a positive test result, compared to just 6.4% in the group at moderate risk with a negative test result, into which no colorectal cancer cases fell. This was a small study with only seven cases of colorectal cancer, the Asia-Pacific Colorectal Screening score may not necessarily be transferable across geography, and qualitative rather than quantitative FIT was employed, but these results show promise of a sizeable added benefit when supplementing risk scoring models with FIT. A Dutch group have performed similar analysis, this time using questionnaire data from 1,112 participants aged 50-75 years, with logistic regression modelling being used to identify significant risk factors in age, family history of colorectal cancer, alcohol intake, smoking status and history, calcium intake and physical activity, along with the quantitative measure of faecal Hb concentration. (Stegeman *et al.*, 2014) Faecal Hb concentration was found to be the most influential of all the variables in the model, but the multivariate risk model including faecal Hb concentration had better sensitivity than screening with faecal Hb concentration alone. Offering colonoscopy to the 102 individuals at highest risk according to the model rather than to the 102 who had a positive FIT result with a cut-off faecal Hb concentration of 10 µg Hb/g faeces would have detected five more cases of advanced neoplasia. However, again, only seven cases of colorectal cancer were detected overall in the cohort. Programme organisers may be discouraged from implementation of a screening strategy based on the results of this paper due to the use of a questionnaire, which may have a negative effect on

screening participation. A simpler model, with easily acquired variables such as age, gender and faecal Hb concentration, would be more desirable for implementation into a national screening programme.

An example of study using only these three variables for risk stratification has emerged from Catalonia, Spain, with retrospective analysis of 3,109 screening participants aged 60-69 years old. (Auge *et al.*, 2014) Multivariate logistic regression analysis revealed age, gender and faecal Hb concentration to be independently associated with advanced neoplasia and 16 risk categories were formed by combining these factors. The highest category - men aged over 60 - 69 years old with faecal Hb concentration above the third quartile (> 177 µg Hb/g faeces) - had an 11.46-fold (95% CI: 7.25 - 18.10) increased probability of advanced neoplasia compared to the lowest risk category of women aged 50-59 years with faecal Hb concentration in the first quartile (20 - 32 µg Hb/g faeces). It would be of interest to consider how these findings translate into an overall score to prioritise colonoscopy towards those at highest risk. Omata *et al*. (2011) collated data from 1,085 asymptomatic Japanese individuals completing both quantitative FIT and colonoscopy as part of a general health check-up. A very low cut-off faecal Hb concentration of 5 µg Hb/g faeces was deemed by the authors to be optimal for screening for significant neoplasia, but it was stated that the sensitivity at this concentration was less than would be desired at just 51% (95% CI: 39 - 62). However, screening accuracy for was improved through use of a scoring system incorporating faecal Hb concentration, age, gender and BMI, aided by a nomogram to facilitate clinical utility. Once more, the authors conceded that further study with a larger sample size was required. It is clear from the results of these studies, however, that there is a strong argument for the use of risk scoring systems incorporating faecal Hb concentration in colorectal cancer screening programmes, although more large

scale studies, in the screening setting, detailing simple methods of implementation, are required.

Further argument in favour of the adoption into colorectal cancer screening programmes of risk-stratification methods using FIT comes from an important study by Chen *et al*., (2011) showing that faecal Hb concentration at first screening can be a predictor of subsequent colorectal neoplasia. A prospective cohort study following up 45,992 screening attendees using a cut-off faecal Hb concentration of 20 µg Hb/g faeces was conducted over a median of 4.39 years and it was found that the incidence of colorectal neoplasia rose from 1.74 per 1,000 person-years for those whose initial screening faecal Hb concentration was equivalent to 0.0 - 3.9 µg Hb/g faeces, up to 7.08 per 1000 person-years in those with a faecal Hb concentration of 16.0 - 19.9 µg Hb/g faeces. They concluded that risk-stratification methods could be applied to screening participants with faecal Hb concentration just below the cut-off faecal Hb concentration used for follow-up investigation, perhaps with a shorter screening interval so as to sooner detect advanced neoplasia presenting as false negative at initial screening. Likewise, those with very low, or undetectable faecal Hb concentration could perhaps be invited for screening at less frequent intervals, owing to the long-preclinical phase associated with disease progression in colorectal cancer.

These studies demonstrate the value of assessing faecal Hb concentration using quantitative FIT and the potential this holds for improvement of clinical outcomes through future adaptations to colorectal cancer screening programmes. This adds to the rapidly growing evidence base supporting the adoption of FIT in colorectal cancer screening programmes to replace the traditionally used gFOBT. FIT exhibit many advantages over gFOBT as a primary test showing superior performance characteristics, particularly for precursor lesions which may, in the long-term, lead to a

reduction in colorectal cancer incidence. FIT may also go some way to reducing the high number of interval cancers associated with gFOBT. The disproportionate number of interval cancers detected in women suggests that the gFOBT may discriminate against women, who have been shown to have lower faecal Hb concentration than men (Fraser & Auge, 2014; Fraser *et al.*, 2014; Kapidzic *et al.*, 2015; McDonald *et al.*, 2012; Symonds *et al.*, 2015b). A solution to address this issue could be sought through adopting individually tailored screening strategies that are possible with the adjustable cut-off faecal Hb concentration afforded by automated FIT. Although FIT have been, until recently, significantly more expensive than gFOBT, this may be offset by their advantages in terms of superior performance characteristics and improvements in test uptake. Indeed, a cost-effectiveness analysis of FIT compared with gFOBT was conducted in France by Berchi *et al*. (2010) shows that comparing direct costs of one round of FIT screening at different cut-off faecal Hb concentration with gFOBT in 20,322 participants completing both tests showed that using a cut-off faecal Hb concentration of 15 µg Hb/g faeces guaranteed more efficient screening than gFOBT, displaying better health outcomes and lower costs.

1.9 Other screening modalities

Although the focus of this thesis is solely on the use of tests for the presence of Hb in faeces, it is important to mention key studies investigating the effectiveness of other screening modalities.

An alternative primary screening test to be considered for identification of participants relevant for referral for colonoscopy is flexible sigmoidoscopy. Flexible sigmoidoscopy allows visual inspection of the distal 40 - 60 cm of the colon with an enema, usually

self-administered, used prior to the examination to cleanse the bowel. Screening programmes can use flexible sigmoidoscopy in the first stage of screening, with referral for colonoscopy made following detection of adenomas. The basis for this strategy comes from evidence showing that adenomas in the distal colon are predictive of the presence of advanced adenomas in the proximal region. Imperiale *et al.* examined colonoscopy results from 1,994 asymptomatic individuals aged over 50 years and found that compared with those with no adenomas or hyperplastic polyps found distally, those who had distal tubular adenomas had a relative risk of advanced proximal neoplasia of 4.0, and 6.7 in those with an advanced distal adenoma. (Imperiale *et al.*, 2000) However, they also observed that about half of the cases of proximal neoplasia would go undetected if colonoscopy was offered only to those with distal lesions. Despite this, flexible sigmoidoscopy is the only screening test other than FOBT proven to reduce mortality in RCT and, unlike gFOBT, has also demonstrated a reduction in colorectal cancer incidence. Three RCT have been conducted to assess the efficacy of flexible sigmoidoscopy screening in reducing colorectal cancer incidence and mortality. The largest of these took place in the UK, where 170,432 participants aged between 55 and 64 years were assigned randomly on a 2:1 basis to a control group and an intervention arm, respectively. (Atkin *et al.*, 2010) Those in the intervention arm were offered a single round of flexible sigmoidoscopy screening. The criterion for colonoscopy after flexible sigmoidoscopy was detection of high-risk adenoma, giving a 5% referral rate. Results after a median follow-up of 11.2 years showed a 23% reduction in colorectal cancer incidence in the group invited, with the effect rising to 33% in those actually attending screening. Likewise, in intention-to-treat analyses, a 31% reduction in colorectal cancer mortality was observed in the intervention arm, rising to 43% in those actually undergoing flexible sigmoidoscopy. The uptake was 71%; however, trial participants had previously indicated via questionnaire that they would participate in colorectal cancer screening, and lower adherence would be expected in the general population. This is perhaps confirmed by

a very recent publication by McGregor *et al.* (2015) reporting uptake to be 43.1% over the first 14 months of the English Bowel Scope Screening programme pilot, which invites adults aged 55 years for a one off flexible sigmoidoscopy. It was concluded from the UK RCT, (Atkin *et al.*, 2010) however, that once-only flexible sigmoidoscopy was a safe and practical screening test offering substantial, lasting protection from colorectal cancer. A pilot study similar to that running in England is now taking place as an adjunct to the Scottish Bowel Screening Programme, offering flexible sigmoidoscopy as a one-off screening modality in those aged 60 years who are due their gFOBT screening round; preliminary results are awaited to assess uptake and clinical outcomes in comparison to the gFOBT/FIT two-tier reflex algorithm. Hoff *et al.* (2009) reported interim results of the Norwegian Colorectal Cancer Prevention (NORCCAP) trial where 55,736 participants aged 55-64 years were randomised to a screening arm offering once only flexible sigmoidoscopy, or a control group. Half of the 6,908 people in the screening arm were also asked to complete a single round of gFOBT testing, with either neoplasia detected with flexible sigmoidoscopy or a positive gFOBT result qualifying participants for full colonoscopy. In contrast to the UK trial, no reduction in colorectal cancer incidence was observed and the trend towards reduced colorectal cancer mortality was non-significant between the screening and control groups. However, a significant reduction in colorectal cancer mortality of 59% was shown when adjusting for attendees. The smaller effect of once only flexible sigmoidoscopy screening compared with the UK trial is likely to be related, at least in part, to the shorter follow-up time of seven years for colorectal cancer incidence and six for colorectal cancer mortality, with the suggestion that the lag period of colorectal cancer development from precursor lesions is longer than this. Another Norwegian RCT by Thiis-Evensen *et al.* (1999) examined the effect of polypectomy on colorectal cancer incidence in 400 participants offered a flexible sigmoidoscopy-based screening programme in 1983 compared with 399 in a control group. After 13 years, both groups were invited for colonoscopy and cancer registrations in the interim period were

examined. An 80% reduction in colorectal cancer incidence was reported in the screening group compared with the control group in the intention-to-screen analysis. This large effect may be attributed to the high uptake (81%) and to the criteria for referral for colonoscopy set as any kind of polyp detected with flexible sigmoidoscopy, with further colonoscopy offered after periods of 2 and 6 years.

As yet, no randomised trial directly comparing the effect on colorectal cancer mortality of flexible sigmoidoscopy with gFOBT or FIT has been conducted. Although the flexible sigmoidoscopy trials appear to show a greater reduction in mortality than that calculated from gFOBT RCT, despite poorer participation rates, overlap exists meaning that no clear superiority is indicated. Some studies are now suggesting that strategies combining FIT and flexible sigmoidoscopy may represent a route to improved screening effectiveness. (Hol *et al.*, 2010)

1.10 Summary

A diverse range of literature covering many aspects of colorectal cancer screening exists, with much knowledge to be gained from this regarding the best use of colorectal cancer screening tests. Most importantly, the use of gFOBT as a primary test has been proven to reduce colorectal cancer mortality. This screening modality carries disadvantages including a high proportion of false negative test results, gender differences in the clinical performance and a lack of specificity for human Hb. The evidence base supporting the adoption of FIT in colorectal cancer screening programmes due to the many advantages they hold over gFOBT is growing rapidly. Although no RCT has been carried out demonstrating FIT to play a role in the reduction of colorectal cancer mortality, evidence supports the thesis that FIT show a particular

superiority over gFOBT for the detection of precursor lesions. This may mean that FIT can enhance the reduction on colorectal cancer mortality seen with gFOBT and, in the longer term, additionally lead to a reduction in colorectal cancer incidence. Up-to-date reviews of the evidence around the adoption of FIT into colorectal cancer screening programmes concluded that FIT offers opportunities for further enhancement to colorectal cancer screening programmes and its use as the test of choice can no longer be denied. (Allison *et al.*, 2014; Young *et al.*, 2015) Further potential for more effective screening programmes comes from the highly favourable option to adjust the cut-off faecal Hb concentration used for referral for colonoscopy afforded to programme organisers with quantitative FIT. A number of studies have demonstrated the advantages of this feature with identification of the cut-off faecal Hb concentration which provides optimal clinical performance, suitable to colonoscopy capacity. A further potential use of this characteristic of quantitative FIT is the incorporation of cut-off faecal Hb concentration that are tailored according to age and gender. Evidence indicates that tests for the presence of Hb in faeces perform less well in women than in men, although FIT may go some way to reducing the gap compared with gFOBT. Also, colorectal cancer risk increases with age. With many other biomarker tests commonly using reference values stratified according to age and gender, surely a similar approach can be incorporated in to screening with quantitative FIT. Such adaptations of the screening programme could improve overall test sensitivity and specificity. This area has not yet been investigated in colorectal cancer screening, representing a gap in the current published research. A further option afforded by quantitative FIT is incorporation of faecal Hb concentration into risk scoring systems. With the screening interval being an important consideration in terms of balancing costs, participation and detection rates, perhaps monitoring changes in faecal Hb concentration across multiple screening rounds could allow participants to be assigned a risk category, where high-risk participants can be invited more frequently, and vice-versa. This imaginative approach has potential to boost cost-effectiveness by diverting resources away from

those consistently showing very low or undetectable faecal Hb concentration and focussing more on those showing fluctuations towards higher faecal Hb concentration, and hence greater colorectal cancer risk. It is clear that more frequent screening invitations to those with faecal Hb concentration closer to the cut-off has the potential to reduce the interval cancer proportion, assuming that a proportion of cases of sub-threshold bleeding are associated with lesions on their way to malignancy before the next screening round. This, again, is an area into which research is scarce and the hypothesis that interval cancer proportion can be reduced using this cost-effective strategy deserves further attention. With missed cancers perhaps the most pressing concern associated with gFOBT screening, the potential for FIT to go some way to addressing the high interval cancer proportion is a crucial area of research interest.

1.11 Thesis aims and objectives

The next five Chapters of this thesis will aim to answer a series of research questions around the relationship between faecal Hb concentration and the risk of significant colorectal neoplasia in the setting of colorectal cancer screening. The main questions being asked in each of these Chapters will be:

- What is the relationship between screening test results with a gFOBT/FIT two-tier reflex algorithm and severity of colorectal neoplasia?
- What is the relationship faecal Hb concentration and severity of colorectal neoplasia?
- What was the faecal Hb concentration of participants who had a negative screening test result and were later diagnosed with interval cancer?

- What was the faecal Hb concentration of participants who had a negative screening test result, then had a positive screening test result at the subsequent screening round?
- Is there an independent trend of increasing faecal Hb concentration with increasing degree of deprivation?

If, in answering these questions, it is shown that faecal Hb concentration can act as an important predictor of colorectal neoplasia, then perhaps the results will call for the properties afforded by quantitative FIT to be used to their maximal potential. This could allow for better detection rates of advanced neoplasia within the boundaries of the resources available to the programme.

The final Chapter will shift focus from screening to the symptomatic population, assessing the potential benefits to be gained from the use of FIT in primary care to aid GP when making decisions with regard to which patients with lower abdominal symptoms should be referred for invasive investigation. With investigative services in the UK being placed under an increasing burden from primary care referrals, a means to reduce the number of referrals without missing clinically important disease is urgently required. With this in mind, the following research questions will be asked in the final results Chapter:

- What is the diagnostic accuracy of quantitative FIT in patients presenting to primary care with colorectal symptoms?
- What is the appropriate cut-off faecal Hb concentration to rule out significant colorectal disease?

If faecal Hb concentration can act as a good rule-in test for colorectal cancer and, more importantly, a good rule-out test for significant colorectal disease in primary care, potential exists for colonoscopy resource to be redirected into screening. This would allow for lowering of the relatively high faecal Hb concentration cut-off used in Scotland, and for more sophisticated adjustment of screening strategy

2. The relationship between results with the guaiac faecal occult blood test/Faecal Immunochemical Test two-tier reflex screening algorithm and severity of colorectal neoplasia

2.1 Introduction

Before investigating possible adaptations to colorectal cancer screening protocols which may be facilitated by the emergence of quantitative Faecal Immunochemical Tests for haemoglobin (FIT), it is first important to improve understanding of the relationship between faecal haemoglobin (Hb) concentration and disease severity. Hb in faeces is strongly associated with colorectal cancer and larger precursor lesions. However, the presence of blood, often termed occult blood, can often also present in individuals with various other gastrointestinal conditions, or indeed in those with no evident pathology detected at colonoscopy, leading to individuals with false positive test results in colorectal cancer screening. The possible mechanisms driving blood loss from the seemingly normal colon represent a poorly understood area. It is vital for the success of any efficient screening programme that the primary test selects a group of participants with positive test results that harbour more cases of the disease of interest than would be found from simply a random selection of the screening population sent forward for invasive bowel visualisation. Measures of test sensitivity and specificity for the detection of advanced neoplasia determine whether or not this crucial objective is being met, but calculation of these characteristics requires the disease status of participants with negative as well as positive screening test results.

This is not readily available when conducting an audit of screening data. Detailed analysis of clinical outcomes of those with positive screening test results alone can, however, offer some valuable insight into the relevance of faecal Hb concentration in the detection of serious colonic lesions.

Correlation of faecal Hb concentration with severity of colorectal neoplasia is now more easily afforded by the emergence of quantitative FIT. However, some countries still use traditional guaiac faecal occult blood test (gFOBT); at the time of preparation of this work, this is still the case in Scotland. Although it became clear during the pilot rounds of the Scottish Bowel Screening Programme that FIT had significant advantages over gFOBT, (Duffy *et al.*, 2011; Fraser, 2011a) qualitative FIT were considerably more costly than gFOBT and their lower analytical detection limit meant that the test positivity rate would be much higher than the ca. 2% that could be sustained by the available colonoscopy resource in Scotland; their use as a first-line test was therefore precluded. However, in order to take advantage of the superior analytical specificity of FIT and to cut down the number of false positive test results obtained with gFOBT, a suggested strategy is the use of gFOBT as a first-line test followed by use of FIT for individuals with positive gFOBT results. (Young *et al.*, 2002) This strategy was investigated (Fraser *et al.*, 2006; Fraser *et al.*, 2007) and the theoretical benefits were attained, at least in the research setting. The approach was described as the two-tier reflex gFOBT/FIT screening algorithm (Fraser *et al.*, 2006) and, because of the positive experience, this was adopted for the Scottish Bowel Screening Programme, in which all individuals aged 50-74 years are invited to participate every two years.

Although no absolute faecal Hb concentration is quantitated, some scope for investigation of the relationship between the concentration of Hb detected in the sample and disease severity is, however, still possible within programmes using gFOBT due to the collection card featuring six 'windows' for the application of faeces, with two samples taken from each of three consecutive bowel movements required. The current protocol within the two-tier gFOBT/FIT screening algorithm sees participants being classified depending on how many of the six windows produce a colour change when hydrogen peroxide is applied to the test card in the laboratory, indicating the presence of Hb with the result determined as either negative (no windows positive), strong positive (five or six windows positive) or weak positive (one to four windows positive). In those deemed weak positive, re-testing using a qualitative FIT, based upon lateral flow immunochromatography, is required to establish a final screening result. It can be sensibly speculated that those with a strong positive gFOBT result have more severe colonic blood loss than those with fewer windows positive in the initial test. It then follows that the prevalence of clinical outcomes detected between groups from the two routes to test positivity can be compared to establish whether or not strong positive test results show higher association with cases of colorectal neoplasia.

With age and gender having been shown to relate to faecal Hb concentration (McDonald *et al.*, 2012), variation in the demographic characteristics of participants following the different routes to test positivity can also be assessed to provide evidence of how this translates to the clinical outcomes detected following a positive gFOBT result. This work may yield findings that offer important insight into the unravelling of some of the unanswered questions related to colorectal cancer screening, such as why women have a higher proportion of interval cancer than men. (Brenner *et al.*, 2012; Gill *et al.*, 2012; Steele *et al.*, 2012) Location of colonic lesions detected in relation to the

route to test positivity can also be analysed; right-sided, and rectal tumours have both been shown to be more prevalent in interval cancer cases than in screen-detected cases. (Gill *et al.*, 2012; Hosokawa *et al.*, 2003; Jensen *et al.*, 1992; Steele *et al.*, 2012; Tazi *et al.*, 1999)

The aims of this Chapter were to determine if more severe disease is detected in those with a strong positive gFOBT result compared with those with an initially weak positive gFOBT result and if there are any trends in positivity and clinical outcomes by gender. With gFOBT still utilised as the primary colorectal cancer screening test in Scotland and elsewhere in the UK, findings of this analysis are important in terms of understanding some of the strengths and weaknesses of the current screening algorithm.

2.2 Materials and methods

All invited were sent a gFOBT kit, which required two samples from each of three faeces collected by cardboard applicator and applied to the six windows of the test card (*hema-screen*, Immunostics Inc, Ocean, NJ, USA). If no windows were positive, the participant was sent an informative letter. If five or six windows were positive, this was described as a strong positive test result and the participant referred for colonoscopy in the NHS Board of residence. If one to four windows were positive, the result was deemed a weak positive and the participant was sent a qualitative FIT kit that required one sample from each of two faeces collected by cardboard applicator and applied to the two windows of the card collection device: in the laboratory, the dried faeces on the tab of the card was placed in a tube containing buffer and qualitative analysis done on

an immunochemical test cassette (*hema-screen SPECIFIC*, Immunostics Inc, Ocean, NJ, USA). The details of this FIT methodology have been described (Allison *et al.*, 2014; Fraser *et al.*, 2007); positive test results are detected at a concentration of 50 ng Hb/ml buffer, equivalent to 50 µg Hb/g faeces. If the test result was positive, the participant was referred for colonoscopy and. Participants with an initial weak positive gFOBT who then had a negative qualitative FIT result were declared negative, not invited for colonoscopy,sent an informative letter and reinvited in two years if still eligible for screening. Some participants did not provide a testable gFOBT due to the kit being expired, incomplete, spoiled by the participant or unused, or having a technical problem or an irresolvable participant identity difficulty; in order to expedite the screening pathway, these were sent a FIT rather than a repeat gFOBT.

All analyses were carried out in the Scottish Bowel Screening Centre Laboratory by trained staff whose major function is to perform faecal test analyses; the Laboratory had a comprehensive total quality management system and was accredited to International Organization for Standardization (ISO) 15189 based standards by Clinical Pathology Accreditation (UK) Ltd.

All results from 01 July 2007 to 30 June 2009 inclusive were examined: this represents the entire fourth round of screening in NHS Tayside. Positive results arose because test results were (1) strong positive gFOBT, (2) weak positive gFOBT followed by a FIT positive test result or (3) an untestable kit being submitted followed by a FIT positive test result. For all positive test results, data for colonoscopy outcomes and pathology were downloaded from the appropriate NHS Tayside clinical IT systems. Data on colonoscopy were collected on the quality of the investigation (quality of preparation, completeness of colonoscopy) and on the results including number, size and

localization of colorectal cancer and adenomas, and whether biopsy was performed. Full pathological data were collected on all excised/biopsy specimens including polyp type, presence or absence of malignancy and, in all adenomas, the severity of dysplasia. Assignment as higher-risk adenoma was ≥3 adenomas, or any adenoma with a maximum diameter ≥10 mm, derived from the recommendation from the British Society of Gastroenterology (Atkin & Saunders, 2002) as used in Scotland. Where participants had more than one diagnosis made, the most serious diagnosis was recorded.

MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software was used for all calculations. Logistic regression analysis was performed to calculate odds ratio for disease outcomes between the different routes to test positivity.

This evaluation was approved by the Scottish Bowel Screening Programme Board and the Caldicott Guardian of NHS Tayside.

2.3 Results

Over the screening round observed, 131,885 people were invited and 73,315 responded, giving an uptake of 55.6%. As seen previously in the Scottish Bowel Screening Programme, uptake was higher in women (60.3%) than in men (53.2%). Of the initial responses 66,957 (91.3%) results were negative. There were 241 (0.3%) strong positive test results, of which 143 (59.3%) were men, 5,230 (7.1%) weak positive test results of which 2,999 (57.3%) were men, and 887 (1.2%) untestable results of which 471 (53.1%) were men. Of the 5,230 with a weak positive test result,

983 (18.8%) went on to have a positive qualitative FIT result. The overall test positivity rate was 1.77%, with a total of 1,301 participants with positive test results. Table 2.1 shows the proportions of participants in each route to test positivity.

The majority of positive test results came about from an individual having a weak positive gFOBT result, followed by a positive qualitative FIT result (75.6%). By all routes, 785 (60.3%) positive test results were found in men. Of the strong positive gFOBT, weak positive gFOBT plus positive FIT and untestable followed by positive FIT result routes, 59.3%, 61.0% and 54.5% respectively were males. There were no significant differences ($p > 0.05$) between the route to test positivity by gender, nor in median age in each route.

Table 2.1. Numbers and proportions of participants in each route to test positivity.

	Total		Men		Women	
	n	%	n	%	n	%
Total positive test results	1,301		785		516	
Strong positive gFOBT results	241	18.5	143	18.2	98	19.0
Weak positive --> positive FIT results	983	75.6	600	76.4	383	74.2
Untestable gFOBT --> positive FIT results	77	5.9	42	5.4	35	6.8

The clinical outcomes of colonoscopy and pathology in the 1,301 participants with positive test results overall, and in the three groups with different routes to test positivity, are shown in Table 2.2. It should be noted that the majority of cases of advanced neoplasia (310/396, 82.2%) were detected in those who had an initial weak positive gFOBT result followed by a positive qualitative FIT. However, this group was 4

times larger than the group with an initial strong positive gFOBT result. A higher proportion of participants in the group with an initial strong positive gFOBT result had a diagnosis of colorectal cancer than any other route to positivity. However, the group with an initial weak positive gFOBT result followed by a positive qualitative FIT had a higher proportion of higher-risk adenoma than any other route to positivity. For completeness, Table 2.2 includes outcomes of those who arrived at their positive screening result with a positive qualtitative FIT following an initial untestable gFOBT. Since this group are not relevant in terms of the current analysis of groups of differing degrees of faecal Hb concentration detected, this group is not included in Table 2.3, which shows the Positive Predictive Value (PPV) for neoplasia within each group, as well as proportions of other clinical outcomes, by gender. Within the group with a strong positive gFOBT result, the PPV for colorectal cancer was higher in women than in men. Table 2.4 shows odds ratios for cancer, higher-risk adenoma and advanced neoplasia, broken down by gender. Adjusted odds ratio for colorectal cancer were significantly higher in those with a strong positive gFOBT than those with a weak positive gFOBT followed by a positive qualitative FIT (2.15, 95% CI: 1.41 – 3.27). This was true for both genders, but adjusted odds ratio were higher for women (2.52, 95% CI: 1.35 – 4.72) than for men (1.78, 95% CI: 1.00 – 3.16).

Table 2.2. Clinical outcomes for participants with positive test results according to route to test positivity.

Outcome	Total (%)	Strong positive gFOBT results (%)	Weak positive gFOBT + positive FIT results (%)	Untestable gFOBT + positive FIT results (%)
Cancer (CRC)	129 (9.9)	38 (15.8)	87 (8.9)	4 (5.2)
Higher-risk adenoma (HRA)	267 (20.5)	39 (16.2)	223 (22.7)	5 (6.5)
Advanced neoplasia (CRC + HRA)	396 (30.4)	77 (32.0)	310 (31.5)	9 (11.7)
Low-risk adenoma (LRA)	145 (11.1)	15 (6.2)	118 (12.0)	10 (13.0)
Total adenoma	409 (31.4)	54 (22.4)	343 (34.8)	15 (19.5)
Total neoplasia(CRC + HRA + LRA)	538 (41.4)	92 (38.2)	428 (43.5)	19 (24.6)
Other outcomes:	763 (58.6)	149 (61.8)	555 (56.5)	58 (75.4)
Hyperplastic polyps	80 (6.1)	15 (6.2)	63 (6.4)	2 (2.6)
Other non-neoplastic pathology*	244 (18.8)	63 (26.1)	166 (16.9)	15 (19.5)
No pathology detected	287 (22.1)	43 (17.8)	218 (22.2)	26 (33.8)
Did not attend	147 (11.3)	38 (15.8)	94 (9.6)	15 (19.5)

* - Other non-neoplastic pathology comprise conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

Table 2.3. Positive Predictive Values (PPV) for colorectal neoplasia according to route to positivity.

	Strong positive gFOBT results		Weak positive gFOBT + positive FIT results	
	n	PPV	n	PPV
Cancer (CRC):				
Total	38	18.7%	87	9.8%
Men	19	16.0%	51	9.5%
Women	19	22.6%	36	10.3%
Higher-risk adenoma (HRA):				
Total	39	19.2%	223	25.1%
Men	30	25.2%	158	29.3%
Women	9	10.7%	65	18.6%
Advanced neoplasia (CRC + HRA):				
Total	77	37.9%	310	34.9%
Men	49	41.2%	209	38.8%
Women	28	33.3%	101	28.9%
Total adenoma (HRA + LRA + unclassified adenoma):				
Total	54	26.6%	340	38.3%
Men	38	31.9%	246	45.6%
Women	16	19.0%	94	26.9%
Total neoplasia (CRC + total adenoma):				
Total	92	45.3%	427	48.1%
Men	57	47.9%	297	55.1%
Women	35	41.7%	130	37.2%
Non-neoplastic pathology* + low-risk adenoma:				
Total	83	40.9%	360	40.5%
Men	50	42.0%	225	41.7%
Women	33	39.3%	135	38.7%
No pathology detected:				
Total	43	21.2%	218	24.5%
Men	20	16.8%	105	19.5%
Women	23	27.4%	113	32.4%

* - Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

Table 2.4. Unadjusted and adjusted odds ratios with 95% confidence intervals (CI) for significant neoplasia in those with a strong positive guaiac faecal occult blood test (gFOBT) result, using those with an initial weak positive gFOBT result as the reference category.

	Strong positive gFOBT result PPV	Weak positive gFOBT + positive FIT result PPV	Odds ratio (95% CI)*	Adjusted odds ratio (95% CI)*,**
Cancer (CRC):	18.7%	9.8%	**2.12 (1.40 - 3.22)**	**2.15 (1.41 - 3.27)**
Men	16.0%	9.5%	**1.81 (1.03 - 3.21)**	**1.78 (1.00 - 3.16)**
Women	22.6%	10.3%	**2.54 (1.37 - 4.71)**	**2.52 (1.35 - 4.72)**
Higher-risk adenoma (HRA):	19.2%	25.1%	0.71 (0.48 - 1.04)	0.72 (0.49 - 1.05)
Men	25.2%	29.3%	0.81 (0.52 - 1.28)	0.83 (0.52 - 1.30)
Women	10.7%	18.6%	0.52 (0.25 - 1.10)	0.52 (0.25 - 1.10)
Advanced neoplasia (CRC + HRA):	37.9%	34.9%	1.13 (0.83 - 1.56)	1.15 (0.84 - 1.59)
Men	41.2%	38.8%	1.11 (0.74 - 1.66)	1.11 (0.74 - 1.67)
Women	33.3%	28.9%	1.23 (0.74 - 2.04)	1.21 (0.73 - 2.03)

* Values in bold represent a statistically significant difference ($p < 0.05$).
** Totals adjusted for age quintile and gender, and for age quintile only for values for men and women.

From the 106 cancers for which staging was available from the strong positive gFOBT result group and the initial weak positive gFOBT then positive FIT result group, Dukes' stage distribution is shown in Table 2.5. In men, 19 (32.2%), 20 (33.9%), 19 (32.2%) and 1 (1.7%) were Dukes' A, B, C1 and C2 respectively: in women, 14 (29.8%), 7 (14.9%), 23 (48.9%) and 3 (6.4%) were diagnosed at these stages. Table 2.6 shows the proportion of cancers in each route which are either early stage (Dukes' stage A or B) or late stage (Dukes' stage C1, C2 or D), with ORs for those with a strong positive gFOBT result compared to those with an initial weak positive gFOBT. Maximum tumour diameter was larger in colorectal cancer cases diagnosed following a strong

positive gFOBT result compared to those with an initial weak positive gFOBT result (39 mm, 95% CI: 33 - 44 v. 31 mm, 95% CI: 27 - 34). Table 2.7 displays the site distribution of colorectal cancer in both routes to test positivity.

Table 2.5. Proportion of cancers at each Dukes' Stage in each route to test positivity.

Dukes' stage	Total		Strong positive gFOBT result		Weak positive gFOBT + positive FIT result	
	n	%	n	%	n	%
A	33	31.1	4	12.5	29	39.2
B	27	25.5	11	34.4	16	21.6
C1	42	39.6	16	50.0	26	35.1
C2	4	3.8	1	3.1	3	4.1

Table 2.6. Proportion of early and late stage colorectal cancer by route to test positivity, with unadjusted and adjusted odds ratios with 95% confidence intervals (CI), using those with an initial weak positive guaiac faecal occult blood test (gFOBT) result as the reference category.

Cancer stage	Strong positive gFOBT result		Weak positive gFOBT + positive FIT result		Odds ratio (95% CI)*	Adjusted Odds ratio (95% CI)*,**
	n	%	n	%		
Early (Dukes' A or B):	16	48.5	47	61.8	1.54 (0.86 - 2.78)	1.56 (0.86 - 2.81)
Men	8	50.0	33	73.3	1.13 (0.51 - 2.50)	1.10 (0.49 - 2.46)
Women	8	47.1	14	45.2	**2.50 (1.01 - 6.18)**	2.47 (0.99 - 6.18)
Late (Dukes' C):	17	51.5	29	38.2	**2.82 (1.54 - 5.17)**	**2.87 (1.55 - 5.29)**
Men	8	50.0	12	26.7	**3.22 (1.29 - 8.07)**	**3.17 (1.26 - 8.02)**
Women	9	52.9	17	54.8	**2.50 (1.11 - 5.65)**	**2.48 (1.09 - 5.66)**

* Values in bold represent a statistically significant difference ($p < 0.05$).

** Totals adjusted for age quintile and gender, and for age quintile only for values for men and women.

Table 2.7. Site distribution of colorectal cancers in each route to test positivity.

Colorectal cancer site*	Total		Strong positive gFOBT result		Weak positive gFOBT + positive FIT result	
	n	%	n	%	n	%
Right-sided	35	29.2	13	35.1	22	26.5
Left-sided	36	30.0	11	29.7	25	30.1
Rectum	49	40.8	13	35.1	36	43.4
Total	120		37		83	

* Right-sided includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.

2.4 Discussion

Analysis of the clinical outcomes of these participants provides some insight into the relationship between degree of colonic blood loss as indicated by route to test positivity, and severity of colorectal disease, with evidence of more severe outcomes in those with a strong positive gFOBT result.

Despite the Scottish Bowel Screening Programme currently being gFOBT-based as the initial test, the majority of participants with a final positive test result were, in fact, from the more analytically sensitive qualitative FIT result. However, those showing evidence of material with peroxidise activity in every faecal sample collected on the initial gFOBT card did display greater evidence of colonic bleeding and therefore, more severe underlying pathology would be expected.

Differences were present between some PPV for neoplasia between the two routes to a positive screening result examined. The PPV for colorectal cancer was higher in those who had a strong positive gFOBT result compared with those who had an initial weak positive gFOBT result. This remained true for women when broken down by gender, but to a lesser extent for menMcDonald *et al.* (2012) had shown evidence in a population in Scottish of women having lower median faecal Hb concentration than men, and this has since been confirmed in other populations worldwide. (Fraser & Auge, 2014; Fraser *et al.*, 2014; Symonds *et al.*, 2015b) It can perhaps be suggested that women with sufficient Hb in the faeces to trigger a strong positive gFOBT result are more likely than men to have serious underlying pathology. The results here would back up this theory.

Interestingly, fewer adenomas were detected in those with a strong positive gFOBT result compared with those who had a weak positive gFOBT then positive FIT result. It was the thesis behind the implementation of undertaking FIT rather than a secondary gFOBT in initial weak positive participants that clinical performance would be improved due to the better analytical sensitivity and specificity of FIT. These results are in keeping with findings that improved test performance with FIT compared to gFOBT is particularly evident for adenoma detection. (Rabeneck *et al.*, 2012) From the data presented here, this was true for men only.

Since the completion of this analysis, similar work has emerged from England showing that it is possible to demonstrate that the risk of colorectal cancer is related to the number of windows testing positive for Hb on a gFOBT card. (Geraghty *et al.*, 2014) colorectal cancer was detected in 21.3% of those with five or six windows showing positive test results compared with 5.9% in those with a weak positive test result ($p <$

0.001). Conversely, fewer intermediate-risk adenomas (3-4 small adenomas or at least one adenoma ≥10 mm) were detected in those with five or six windows positive test results than in those with a weak positive test result (9.0% v. 13.6%), termed “unclear” in the NHS Bowel Screening Programme in England. This mirrors the findings presented here in the Scottish population.

In addition to having a greater proportion of colorectal cancer, odds ratio produced from logistic regression analysis showed that those in the strong positive gFOBT result group were also more than twice as likely to have late stage colorectal cancer as those with an initial weak positive gFOBT followed by a positive FIT result, and only four of the 32 staged colorectal cancers (12.5%) in this group were Dukes’ stage A compared to 39.2% in the initial weak positive gFOBT then positive FIT result group.

The fact that no substantial differences were detected in colorectal cancer site distribution between the two routes to positivity may indicate that there is no general difference in the severity of colonic blood loss depending on colorectal cancer location, and further logistic regression analysis found this to be true when controlling for tumour size and Dukes’ stage. It does seem significant, however, that strong positive test results were triggered by malignant tumours with a larger mean size than those in the initial weak positive test result group. This indicates that larger lesions are associated with an elevated degree of blood loss into the colon.

The differences seen in these results between men and women raise some concern. Although men had a higher test positivity rate than women, the overall PPV for colorectal cancer was higher in women than men in this cohort (10.7% v. 8.9%).

Moreover, in both routes to positivity examined, overall Dukes' stages were later in women in than in men, with 32.8% of all colorectal cancer in men being late stage in contrast to the 54.2% in women. Since it has been found that a higher proportion of colorectal cancer in women arise as interval cancer, (Brenner *et al.*, 2012; Gill *et al.*, 2012; Steele *et al.* 2012) the use of an identical screening strategy for both genders perhaps does not provide equality in terms of meeting the primary aim of colorectal cancer screening i.e., to reduce colorectal cancer mortality through early detection and removal of precursor lesions. Men did, however, have more adenomas of all types than did women, including having than double the PPV for higher-risk adenoma than in women in the strong positive gFOBT result group. Low-risk adenoma, however, are less likely to be bleeding enough to trigger a positive test and therefore discovery of such lesions at colonoscopy can be deemed largely incidental. These findings, therefore, probably simply reflect the greater prevalence of adenoma in men in the general population.

Although the cohort came from the third incidence screening round in NHS Tayside, some participants will have been taking part in colorectal screening for the first time, such as those aged 50 years and new residents of the NHS Tayside catchment area. However, the IT system used by the Scottish Bowel Screening Programme does not allow for a bulk download of the screening round status of a cohort of participants and these data would require collection on an individual basis. Therefore, owing to the size of the cohort being investigated, it was not feasible to perform analysis according to prevalence or incidence screening round. Since the yield of disease is higher in those taking part in prevalence screening, this may represent a limitation to this work, although the majority of those invited over the time period used will be taking part in incidence screening.

Despite the fact that the relationship between faecal Hb concentration and severity of disease cannot be directly assessed in this investigation, the comparisons made between participants in the two different routes to positivity does provide a useful basis for further study on this topic. The finding that those with strong positive gFOBT result are more likely to have colorectal cancer, and moreover, colorectal cancer with a worse prognosis, gives general support of the existence of this relationship. Furthermore, gender differences in clinical outcomes between the two routes to positivity appear to add weight to the questions being asked regarding the use of a single cut-off faecal Hb concentration for men and women. This requires further study.

Examination of the relationship between faecal Hb concentration obtained using quantitative FIT and the underlying pathology responsible is the next logical step in this work.

3. The relationship between faecal haemoglobin concentration and severity of colorectal neoplasia

3.1 Introduction

In the previous Chapter, evidence was presented showing that participants who display increased colonic bleeding have more severe disease. This came from analysis of clinical outcomes according to the number of positive windows on the guaiac faecal occult blood test (gFOBT) card in a large cohort of screening participants. However, with gFOBT now deemed obsolete, (Young *et al.*, 2012) what would be far more enlightening would be a detailed analysis of how estimation of actual faecal haemoglobin (Hb) concentration correlates to pathology detected by the gold standard colonoscopy; this can be achieved with quantitative Faecal Immunochemical Tests for haemoglobin (FIT).

A clearer understanding of how different features of colorectal pathology are associated with varying degrees of colonic blood loss can guide screening programme organisers when selecting an appropriate cut-off faecal Hb concentration. With the key aim of screening being the early detection and removal of precursor lesions, identifying those adenomas at greatest risk of progressing towards malignancy, rather than small polyps that are unlikely to ever progress, would be useful to provide an efficient programme that limits exposure of its participants to unnecessary, invasive

colonoscopy. It is known that some histopathological features of adenoma are associated with a higher risk of progression to malignancy. These include adenomas displaying villous histology rather than solely tubular features and those showing evidence of high-grade dysplasia. (Lieberman *et al.*, 2012) Evidence showing that such characteristics are associated with elevated faecal Hb concentration in comparison to that of lower risk pathology would provide important backing to the argument that faecal Hb concentration is a strong predictor of colorectal cancer risk.

Few previous studies have demonstrated that faecal Hb concentration increases as disease becomes more serious, from the normal colon through low- and high-risk adenomatous polyps, to invasive colorectal cancer. (Auge *et al.*, 2014; Ciatto *et al.*, 2007; Hol *et al.*, 2009; Kovarova *et al.*, 2012; Launoy *et al.*, 2005; Levi *et al.*, 2007; Rozen *et al.*, 2009a) The results of these studies were reviewed in detail in the Introduction to this work. To summarise, the evidence shows that those with advanced neoplasia have significantly higher median faecal Hb concentration than those with less severe findings at colonoscopy. (Auge *et al.*, 2014; Ciatto *et al.*, 2007; Hol *et al.*, 2009; Kovarova *et al.*, 2012; Levi *et al.*, 2007) Liao *et al*. (2013) performed multiple linear regression analysis to reveal a significant association between increasing faecal Hb concentration in seven ordinal scales and severity of neoplasia, from adenoma, to advanced adenoma, to colorectal cancer, adjusted for age and gender in an average-risk Taiwanese population. Launoy *et al.*, (2005) using French colorectal cancer screening programme data, reported colonoscopy outcomes within increasing ranges of faecal Hb concentration. The authors found that the majority of those in the lowest faecal Hb concentration categories did not have neoplasia, whereas 65.2% of those with a faecal Hb concentration above the traditionally used cut-off concentration of 100 ng Hb/ml buffer, or 20 µg Hb/g faeces, had either colorectal cancer or a large adenoma. Indeed, it has been suggested in expert comment that there may be a

continuum of increasing risk as faecal Hb concentration increases from zero. (Fraser, 2011b)

Varying median faecal Hb concentration according to different adenoma characteristics has also been documented, with villous or serrated lesions, and adenomas displaying high-grade dysplasia associating with higher faecal Hb concentration than adenomas with less severe characteristics. (Ciatto *et al.*, 2007; Levi *et al.*, 2007) However, an overriding finding was that lesion size was a strong influencing factor in these results. This would suggest that it is not so much the detailed histopathology of the lesion that determines the degree of colonic blood loss, but more simply that larger lesions are more susceptible to damage to their vasculature by passing faeces, possibly as it is more formed.

Mixed evidence exists surrounding the relationship between faecal Hb concentration and lesion site. A very recent study by van Doorn *et al.* (2015) reported that a finding of higher mean faecal Hb concentration in lesions found in the distal colon compared to those found proximally was not independent of other factors including lesion size and polyp morphology. Levi *et al*. (2007) observed no variation in faecal Hb concentration between proximally and distally located advanced adenoma, whereas Ciatto *et al.* (2007) found that although left-sided adenomas were larger than those in the right colon, left-sidedness did show *independent* association with increasing faecal Hb concentration in adenomas. This was speculated by the authors to be due to faeces in this section of the colon being better formed and therefore having a greater mechanical effect on any polyps with which the faces comes into contact. Further data are required to address the issue of the efficacy of FIT in detecting neoplasia from different regions of the colon.

The existing evidence on the relationship between faecal Hb concentration and clinical outcomes is limited and no clear consensus exists on variation in faecal Hb concentration according to the location in the colon of neoplasia. With some publications showing that a higher proportion of interval cancers are right-sided than screen-detected colorectal cancer, better understanding of any differences in faecal Hb concentration according to lesion site could be important to understanding why some cancers are missed by screening. To date, no studies investigating the relationship between faecal Hb concentration and clinical outcomes have been conducted where a high cut-off faecal Hb concentration was set for test positivity within a colorectal cancer screening programme. It would be valuable for countries, such as the Scotland and the rest of the UK, to have such results at their disposal, this time against a backdrop of limited colonoscopy capacity. In addition, by further documenting that participants with elevated faecal Hb concentration are at significantly greater risk than those with low faecal Hb concentration, the potential of faecal Hb concentration to add to the performance of risk scoring models cannot be ignored.

With this in mind, the aim of this section was to investigate the relationship between faecal Hb concentration and disease severity in a cohort of average risk participants completing a single sample quantitative FIT within the Scottish Bowel Screening Programme.

3.2 Materials and methods

From 1 July 2010 to 12 January 2011, The Scottish Bowel Screening Programme conducted an evaluation of using quantitative FIT in two of the 14 NHS Boards in Scotland. Termed the 'FIT as a First-Line Test' evaluation, this was conducted to determine clinical outcomes in a cohort screened with FIT at a high cut-off faecal Hb concentration to maintain colonoscopy demand within the limits of the available resource, and compare these outcomes with control groups completing the gFOBT/FIT two-tier reflex algorithm. The evaluation also aimed to assess any improvements in uptake that may occur when screening with FIT, the overall planning and delivery of FIT screening, analytical reproducibility of faecal Hb concentration measurements in the laboratory, any technical issues arising with FIT, and consistency and quality of reagents. The results of the evaluation have been described elsewhere. (Steele *et al.*, 2013)

For the 'FIT as a First-Line Test' evaluation, all eligible participants in the Scottish Bowel Screening Programme, aged 50–74 years, resident in NHS Tayside and NHS Ayrshire & Arran, were sent a quantitative FIT kit pack containing an invitation letter, a booklet on colorectal cancer and a thin card wallet with written and pictorial instructions for sample collection containing a single faecal sample collection device (Eiken Chemical Co., Ltd, Tokyo, Japan) and a small zip-lock plastic bag with integral absorbent material and a foil mailing pouch for device return. On return to the Laboratory, receipt of a specimen was captured electronically by the Scottish Bowel

Screening System through a barcode. Gender and age were derived from the bar-coded CHI number, the 10-digit number used through the health care system in Scotland. The barcode labelled specimen collection tubes were assayed for faecal Hb concentration on one of two OC-Sensor Diana automated immunoturbidimetry analysers (Eiken Chemical Company, Tokyo, Japan). Analyses were carried out by trained staff: the laboratory had a comprehensive total quality management system and International Organization for Standardization (ISO) 15189 based standards by Clinical Pathology Accreditation (UK) Ltd.

It has been proposed that all FIT data be expressed as µg Hb/g faeces and a multiplier can be applied to each analytical system:(Fraser *et al.*, 2012) for the OC-Sensor Diana, ng Hb/ml buffer data are multiplied by 0.2. Samples with results above the upper analytical limit were not diluted and re-assayed but reported as greater than that upper concentration limit.

All participants with faecal Hb concentration less than 80 µg Hb/g faeces were reported as having a negative screening result and sent an informative letter. All participants with faecal Hb concentration equal to or above 80 µg Hb/g faeces were reported as having a positive screening test result and sent an informative letter, the general practitioner notified and the individual referred to their NHS Board for colonoscopy. The cut-off faecal Hb concentration was chosen based on an estimate of approximately 2% test positivity to mimic the positivity rate of the existing Screening Programme, selected to match the available colonoscopy resource. Data for colonoscopy outcomes and any subsequent pathology were downloaded from the appropriate NHS Tayside and NHS Ayrshire & Arran clinical IT systems. Data on colonoscopy were collected on the quality of the investigation (quality of preparation, completeness of colonoscopy)

and on the number, size, and localisation of colorectal cancer and adenomas. Full pathological data were collected on all excised/biopsy specimens including polyp type, presence or absence of malignancy, Dukes' stage of any colorectal cancer and, in all adenoma, the severity of dysplasia. Lesion size was recorded from pathology reports except when removed piecemeal, when colonoscopy measurement was used. Right-sided location of colorectal cancer was defined as cancer detected in the region of the colon up to and including the splenic flexure, left-sided as the region thereafter up to the recto-sigmoid junction, and rectal cancer as cancer located at either the recto-sigmoid junction or in the rectum.

Faecal Hb concentration was collated into clinical outcome groups according to most serious diagnosis. Faecal Hb concentration from those with adenoma were further grouped according to characteristics relating to their most serious lesion: size (small, a maximum dimension of <10 mm or large, ≥10 mm), degree of dysplasia (high-grade dysplasia or low-grade dysplasia), villous nature (presence or absence) and location as already defined for colorectal cancer. MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software was used for all calculations and to generate distribution graphs. The Mann–Whitney U test was used for comparison between the groups and median lesion size. Probability of $p \leq 0.05$ was considered significant.

For reasons explained in the Discussion section of Chapter 2 (The relationship between results with the guaiac faecal occult blood test/Faecal Immunochemical Test two-tier reflex screening algorithm and severity of colorectal neoplasia), it was not feasible to separate those participating in prevalence screening from incidence screening. This is also relevant for Chapters 4-6.

3.3 Results

Table 3.1 summarises the number of participants in the study and their clinical outcomes (shown as the most serious diagnosis), using those with completed investigations as the denominator for percentages of each outcome found. Those undergoing colonoscopy had a mean age of 62.8 years and 56.4% were men. Overall, 28.1% of participants undergoing colonoscopy had advanced neoplasia; this was significantly higher in men at 32.5% compared with 22.3% in women ($p < 0.01$).

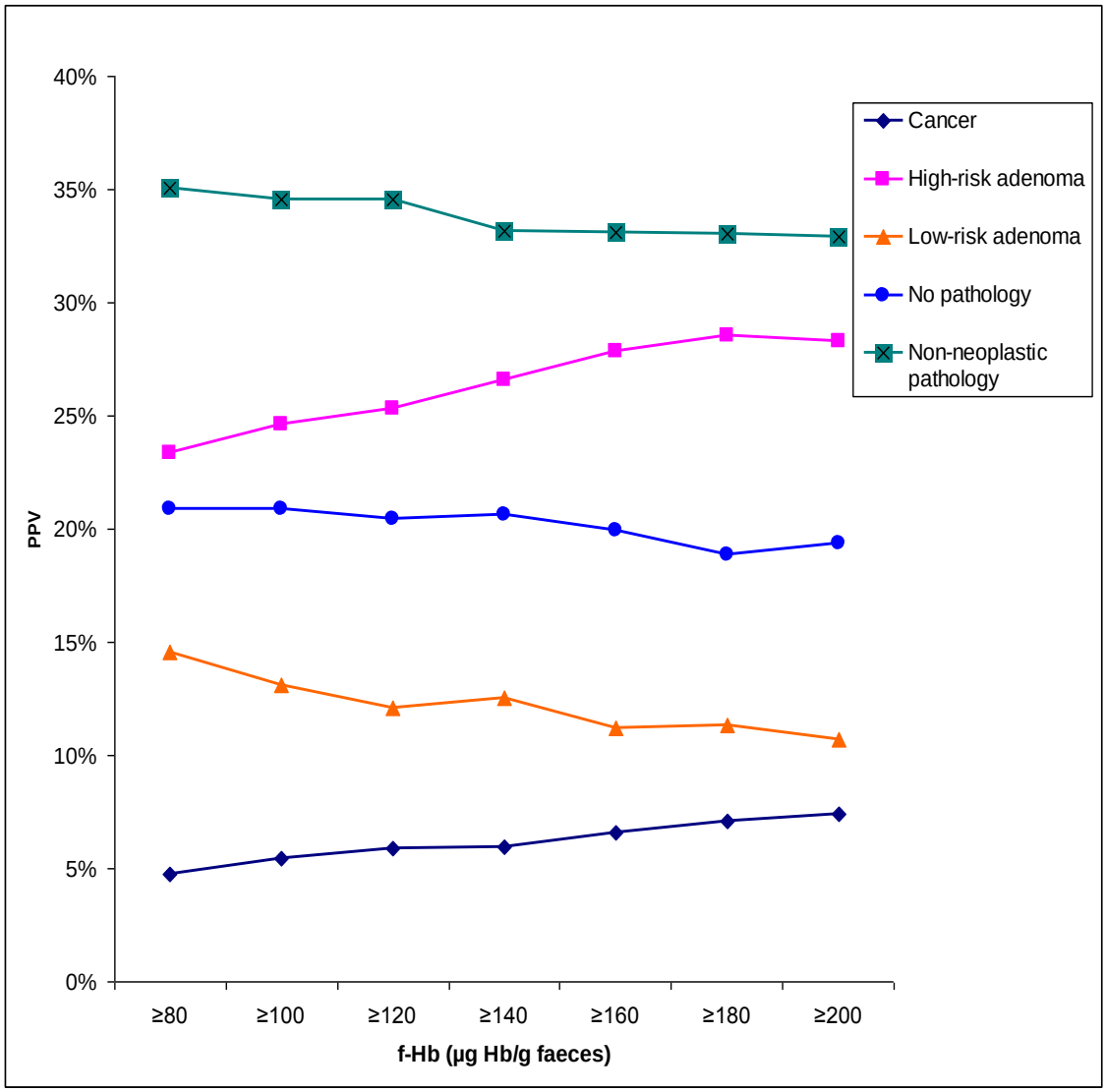
Figure 3.1 displays the proportion of participants with colorectal cancer, higher-risk adenoma, low-risk adenoma, other pathology and no pathology with increasing faecal Hb concentration. The percentage of participants with colorectal cancer rose from 4.8% in all of those with faecal Hb concentration above the cut-off for test positivity of 80 µg Hb/g faeces, to 7.4% in those with faecal Hb concentration at the upper analytical limit of over 200 µg Hb/g faeces. Combining colorectal cancer and higher-risk adenoma gives a Positive Predictive Value (PPV) for advanced neoplasia of 28.1% at 80 µg Hb/g faeces, while 35.7% of those with a faecal Hb concentration of over 200 µg Hb/g faeces had advanced neoplasia. The majority of cases of advanced neoplasia were associated with faecal Hb concentration of over 200 µg Hb/g faeces, with 74.4% of participants with colorectal cancer and 58.1% of those with higher-risk adenoma having faecal Hb concentration above this upper limit reported by the analyser. This compared to 35.3% of those with low-risk adenoma.

Table 3.1. Study participants and clinical outcomes, by gender.

	Total		Men		Women	
	n	%	n	%	n	%
Study participants:						
Invited	66,036		32,245		33,791	
Responders	38,723	43.5	18,057	56.0	20,666	61.2
Positive (faecal haemoglobin concentration ≥ 80 µg Hb/g faeces):	942	2.4	532	2.9	410	2.0
Completed investigations	818	86.8	461	86.7	357	87.1
Declined colonoscopy/unfit	124	13.2	71	13.3	53	12.9
Clinical outcomes:						
Cancer (CRC)	39	4.8	23	5.0	16	4.5
Higher-risk adenoma (HRA)	191	23.3	127	27.5	64	17.9
Advanced neoplasia (CRC + HRA)	230	28.1	150	32.5	80	22.4
Low-risk adenoma (LRA)	119	14.5	77	16.7	42	11.8
Unclassified risk adenoma	2	0.2	1	0.2	1	0.3
Total adenoma	312	38.1	205	44.5	107	30.0
Total neoplasia (CRC + total adenoma)	351	42.9	228	49.5	123	34.5
Inflammatory bowel disease	47	5.7	30	6.5	17	4.8
Diverticular disease	106	13.0	43	9.3	63	17.6
Haemorrhoids	72	8.8	38	8.2	34	9.5
Miscellaneous pathology*	9	1.1	3	0.7	6	1.7
No pathology detected	171	20.9	81	17.6	90	25.2

* Includes four participants with angiodysplasia, two with a rectal ulcer, one with an anal fissure, one with severe melanosis coli and one with intestinal parasite.

Figure 3.1. Positive Predictive Values (PPV) for clinical outcomes with rising faecal haemoglobin concentration (f-Hb).



Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

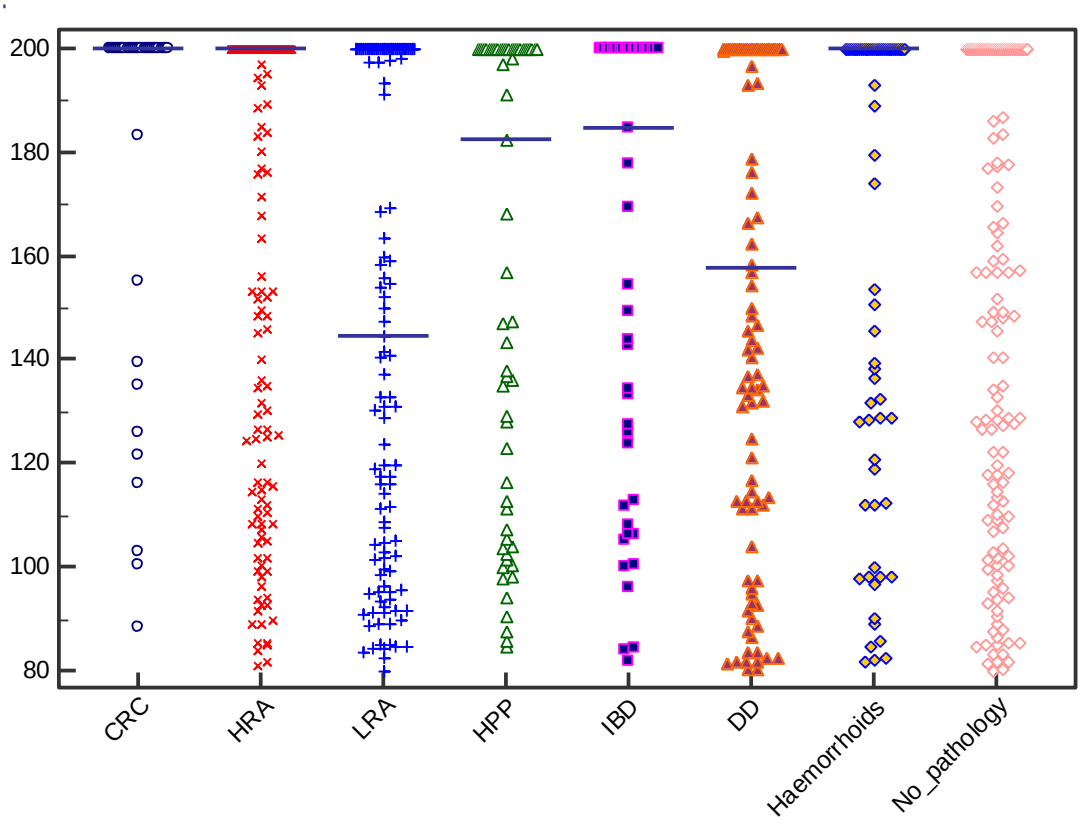
Table 3.2 shows the median faecal Hb concentration for various clinical outcomes with 95% confidence intervals (CI) and interquartile ranges (IQR). Although there is much overlap amongst clinical outcomes and wide CI, median faecal Hb concentration in those with colorectal cancer was statistically significantly higher than that of all other outcomes apart from higher-risk adenoma (p = 0.08). Those with higher-risk adenoma

had median faecal Hb concentration that was higher than in those with low-risk adenoma, hyperplastic polyps, diverticular disease and those with no pathology detected (all $p < 0.03$), but not significantly different to those with inflammatory bowel disease or haemorrhoids (both $p > 0.05$). There was no statistically significant difference between the low-risk adenoma group and those without neoplasia ($p = 0.06$). Median faecal Hb concentration in all participants with advanced neoplasia diagnosed was very highly significantly greater than in those with all other outcomes ($p < 0.0001$). Figure 3.2 illustrates distribution of individual faecal Hb concentration measurements for different clinical outcomes.

Table 3.2. Median faecal haemoglobin concentration (f-Hb) according to clinical outcome with 95% confidence intervals (CI) and interquartile ranges (IQR).

Clinical outcomes	n	Median f-Hb (µg Hb/g faeces)	95% CI	IQR
Cancer	39	200	200 – 200	187 – 200
Higher-risk adenoma	191	200	200 – 200	133 – 200
Low-risk adenoma	119	145	125 – 167	102 – 200
Hyperplastic polyps	62	175	136 – 200	107 – 200
Inflammatory bowel disease	47	184	137 – 200	116 – 200
Diverticular disease	106	158	139 – 198	113 – 200
Haemorrhoids	72	200	147 – 200	125 – 200
No pathology detected	171	170	150 – 200	115 – 200

Figure 3.2. Distribution of faecal haemoglobin concentration (f-Hb) according to clinical outcome.



Horizontal bars represent median f-Hb.

CRC = colorectal cancer, HRA = higher-risk adenoma, LRA = low-risk adenoma, HPP = hyperplastic polyps, IBD = inflammatory bowel disease, DD = diverticular disease.

Table 3.3 shows median faecal Hb concentration according to Dukes' stage, cancer site and cancer type. The differences in median faecal Hb concentration between different stages were not statistically significant, nor were the differences in faecal Hb concentration by tumour site. Of the 39 participants with colorectal cancer, 29 had faecal Hb concentration of more than 200 µg Hb/g faeces and 10 had faecal Hb concentration that could be measured within the analytical working range of 0 – 200 µg

Hb/g faeces. Those within the analytical working range included four participants with a confirmed polyp cancer (Dukes' stage A), two non-polyp Dukes' A cancers, three Dukes' B and one Dukes' stage C1 rectal cancer with f Hb of 88 µg Hb/g faeces. In all, 36 of the colorectal cancer cases had staging available and 12 (six polyp; 27.3%), 11 (33.3%), 12 (36.4%) and 1 (3.0%) colorectal cancer cases were Dukes' A, B, C1 and C2, respectively. Figure 3.3 shows the distribution of faecal Hb concentration according to Dukes' stage.

Table 3.3. Median faecal haemoglobin concentration (f-Hb) of cancers, with 95% confidence intervals (CI) and interquartile ranges (IQR) and significant differences indicated by the p-value in bold.

	n	Median f-Hb (µg Hb/g faeces)	95% CI	IQR	p-value
All cancers	39	200	170 – 193	187 – 200	
Dukes' stage:					
A or B	20	200	160 – 200	147 – 200	0.133
C or higher	13	200	200 – 200	200 – 200	
Tumour site*:					
Right-sided	12	200	200 – 200	200 – 200	0.410
Left-sided	13	200	163 – 200	172 – 200	
Rectum**	14	200	125 – 200	126 – 200	
Cancer type:					
Polyp	6	155	104 – 200	116 – 200	**0.039**
Other	28	200	200 – 200	200 – 200	

* Right-sided includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.
**No significant difference in median f-Hb between rectal cancer and right-sided cancers (p = 0.3566) or between rectal and left-sided cancers (p = 0.9079)

Figure 3.3. Distribution of faecal haemoglobin concentration (f-Hb) according to Dukes' stage of colorectal cancer.

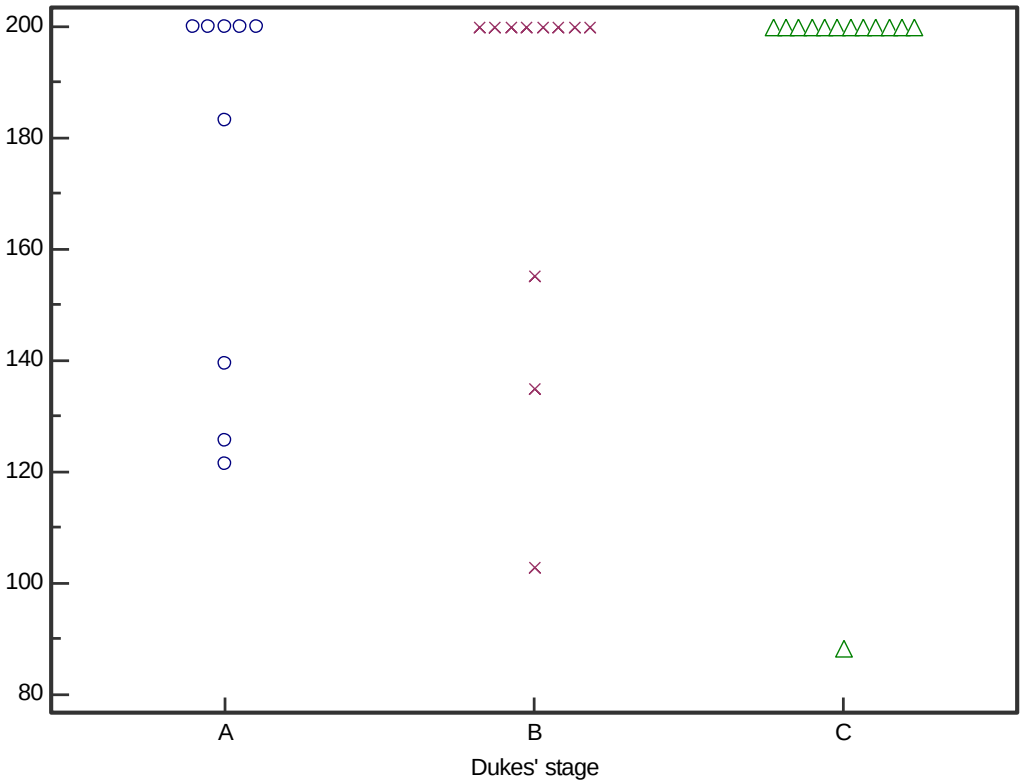


Table 3.4 further classifies those with an adenoma detected according to different polyp characteristics, along with the median faecal Hb concentration and p-values for comparison between these features. Figure 3.4 shows the distribution of faecal Hb concentration according to the different adenoma characteristics examined. Statistically significant differences in median faecal Hb concentration were detected between adenomas classed as large adenomas (≥ 10 mm maximum diameter) and small adenomas (< 10 mm), and between adenomas displaying high-grade dysplasia and those with low-grade dysplasia. Although faecal Hb concentration in those who had an adenoma with a villous component was higher than in non-villous adenoma, this was not statistically significant (p = 0.07). The higher median faecal Hb concentration associated with adenomas situated in the distal colon (beyond the

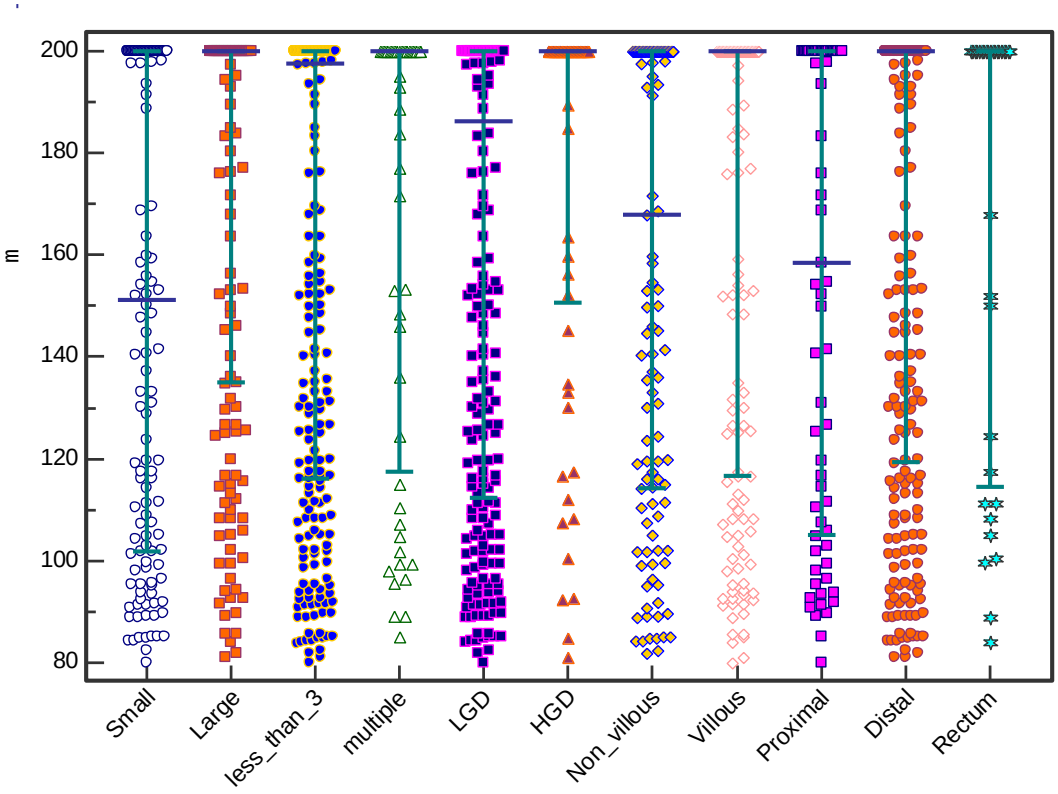
splenic flexure) compared with those more proximal did not reach statistical significance (p = 0.08). No significant difference in faecal Hb concentration was found between those with multiple adenomas (defined as ≥3) and those with only one or two adenomas (p = 0.64).

Table 3.4. Median faecal haemoglobin concentration (f-Hb) according to adenoma characteristic, with 95% confidence intervals (CI) and interquartile ranges (IQR) and significant differences indicated by the p-value in bold.

Adenoma characteristic	n	Median f-Hb (µg Hb/g faeces)	95% CI	IQR	p-value
Small (< 10 mm max. diameter)	134	151	130 – 169	102 – 200	**<0.0001**
Large (≥ 10 mm max. diameter)	174	200	200 – 200	135 – 200	
< 3 adenomas detected	261	198	164 -200	116 – 200	0.592
≥ 3 adenomas detected	51	200	154 – 200	117 – 200	
Low-grade dysplasia	252	186	154 – 200	112 – 200	**0.009**
High-grade dysplasia	57	200	200 – 200	152 – 200	
Non-villous	123	168	142 – 200	114 – 200	0.092
Villous component	156	200	184 – 200	117 – 200	
Right-sided*	67	158	127 – 200	105 - 200	0.077
Left-sided*	209	200	180 – 200	120 - 200	
Rectum*	32	200	150 – 200	115 - 200	

* Right-sided includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.

Figure 3.4. Distribution of faecal haemoglobin concentration (f-Hb) according to adenoma characteristics.



Horizontal bars represent median f-Hb and error bars show interquartile range.

LGD = low-grade dysplasia, HGD = high-grade dysplasia,

The significant difference in faecal Hb concentration seen between high-grade dysplasia and low-grade dysplasia adenomas was not evident when holding size constant, with no difference between small (< 10 mm) low-grade dysplasia and high-grade dysplasia adenomas ($p = 0.88$), or between large (≥ 10 mm) low-grade dysplasia and high-grade dysplasia adenomas ($p = 0.09$). Multiple regression analysis including size, degree of dysplasia, presence or absence of villous component, site and number of adenomas as categorical explanatory variables showed adenoma size was the only characteristic to be significantly related to faecal Hb concentration ($p < 0.0001$; all other variables $p > 0.1$).

Table 3.5 shows the median size of lesions in those with hyperplastic polyps, adenoma or colorectal cancer detected and p-values for comparisons, with significant differences in bold. Significantly larger median lesion size was seen with any adenoma compared with hyperplastic polyps (HPP), higher-risk adenoma compared with low-risk adenoma, high-grade dysplasia compared with low-grade dysplasia adenomas, villous compared with non-villous adenomas, and more advanced cancers compared with polyp cancers. In addition, all cancers were significantly larger than higher-risk adenoma ($p < 0.0001$) and indeed any adenoma ($p < 0.0001$). Adenomas in the distal colon were larger than in the proximal region, in contrast to cancers, which were larger proximally (both $p < 0.05$). No significant differences were seen in the median size of neoplastic lesions detected in the rectum compared with elsewhere in the colon.

Table 3.6 shows the results of multivariate logistic regression on categories of faecal Hb concentration, gender and age to give odds ratios for colorectal cancer and advanced neoplasia in those undergoing colonoscopy. Those with faecal Hb concentration above the upper analytical limit were significantly more likely to have colorectal cancer than those in the lowest category examined (80 - 119 µg Hb/g faeces), independent of age and gender; the same was true for advanced neoplasia.

Table 3.5. Comparison of median lesion size with 95% confidence intervals (CI) according to most severe lesion detected at colonoscopy.

	n*	Median lesion size (mm)	95% CI	Minimum (mm)	Maximum (mm)	p-value
Hyperplastic polyps	38	3	3 - 5	1	16	
All adenoma	307	10	10 - 11	1	85	**<0.0001**
Low-risk adenoma	118	6	4 - 7	1	9	
Higher-risk adenoma	189	14	12 - 15	3	85	**<0.0001**
Adenoma Histology:						
Low-grade dysplasia	250	10	9 - 10	1	50	
High-grade dysplasia	56	14	12 - 17	3	85	**<0.0001**
Non-villous	127	7	6 - 8	1	35	
Villous	154	13	12 - 15	2	85	**<0.0001**
Adenoma site:**						
Right-sided	66	9	7 - 10	1	85	
Left-sided	208	11	10 - 12	2	50	**0.008**
Rectum	32	10	8 - 10	3	30	
Cancer	37	30	25 - 35	7	90	
Dukes' Stage:						
A or B	22	26	25 - 34	7	55	
C or higher	13	35	27 - 42	22	50	0.161
Tumour site:**						
Right-sided	12	35	31 - 46	25	55	
Left-sided	13	25	17 - 29	7	45	**0.005**
Rectum	12	30	24 - 47	18	90	
Cancer type:						
Polyp	5	18	-	7	26	
Other	27	35	25 - 44	9	55	**0.003**

* Not all lesions had a definitive size recorded, therefore some totals differ from those reported elsewhere.

* Right-sided includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.

Table 3.6. Multivariate logistic regression of faecal haemoglobin concentration (f-Hb) category, gender and age quintile with odds ratios with 95% confidence intervals (CI) for colorectal cancer (CRC) and advanced neoplasia (AN). Significant OR shown in bold.

Variable	n	%	Total CRC	% with CRC	Odds ratio (95 % CI)	Total AN	% with AN	Odds ratio (95% CI)
f-Hb category (µg Hb/g faeces):								
80.0 – 119.9	219	28.0	4	1.8	1.00	42	19.2	1.00
120.0 – 159.9	137	17.5	5	3.6	1.76 (0.46 – 6.72)	30	21.9	1.14 (0.67 – 1.93)
160.0 - 199.9	63	8.0	1	1.6	0.82 (0.09 – 7.54)	17	27.0	1.60 (0.83 – 3.09)
≥ 200.0	364	46.5	29	8.0	**4.29 (1.48 – 12.42)**	141	38.7	**2.33 (1.57 – 3.47)**
Gender:								
Men	461	56.3	23	5.0	1.00	150	32.5	1.00
Women	358	43.7	16	4.5	0.97 (0.50 – 1.88)	80	22.3	0.62 (0.45 – 0.85)
Age quintile (years):								
50 - 54	128	15.6	6	4.7	1.00	32	25.0	1.00
55 - 59	169	20.6	3	1.8	0.37 (0.09 – 1.52)	39	23.1	0.90 (0.52 – 1.56)
60 - 64	155	18.9	6	3.9	0.82 (0.26 – 2.64)	47	30.3	1.30 (0.76 – 2.23)
65 - 69	184	22.5	11	6.0	1.24 (0.44 – 3.46)	60	32.6	1.42 (0.85 – 2.38)
≥ 70	183	22.3	13	7.1	1.71 (0.62 – 4.66)	52	28.4	1.26 (0.74 – 2.12)

3.4 Discussion

A wide distribution of faecal Hb concentration exists above the cut-off selected of 80 µg Hb/g faeces, with considerable overlap between groups with different clinical outcomes. However, there is evidence of a relationship between increasing faecal Hb concentration and stage in progression of colorectal neoplasia. In addition to a significantly higher median faecal Hb concentration in participants with any neoplasia detected compared with those with no, or non-neoplastic pathology, increasing faecal Hb concentration is also associated with greater severity of the lesion amongst those with neoplasia detected.

As might be expected, median faecal Hb concentration was significantly higher in participants with higher-risk adenoma compared to those with low-risk adenoma. Furthermore, median faecal Hb concentration in those with low-risk adenoma was perhaps surprisingly low in comparison with those with no neoplasia detected; lower in fact than in those with hyperplastic polyps (although not statistically significant). Indeed, previous studies have found relatively low faecal Hb concentration in non-advanced adenomas, with most giving a concentration below 15 µg Hb/g faeces. (Levi *et al.*, 2007) This is a significant observation, bearing in mind that only a small number of all adenomas will ever progress to malignancy. The ability of faecal Hb concentration to distinguish between those with small low-risk adenoma, and larger lesions more likely to associate with a greater degree of dysplastic change is useful in avoiding overdiagnosis in those with harmless polyps. The repeat nature of colorectal cancer screening with tests for haemoglobin (Hb) in faeces provides opportunities for lesions that do undergo further dysplastic change to be detected at a later screening round, although this does rely on consistent participation.

To further investigate the significant difference in median faecal Hb concentration between higher-risk adenoma and low-risk adenoma, more detailed examinations were made, with participants grouped by the two adenoma characteristics used in this evaluation to determine risk classification: size and number. The fact that a higher median faecal Hb concentration was observed in participants with a larger adenoma compared with those with a small adenoma, but not when comparing participants who had multiple adenomas with those with only one or two adenomas detected, demonstrates that the difference between the higher-risk adenoma and low-risk adenoma group can be attributed to lesion size. Indeed, the median maximum dimension of higher-risk adenoma was over double that of low-risk adenoma and statistically significantly greater.

The findings here confirm those of earlier work (Ciatto *et al.*, 2007; Levi *et al.*, 2007) and are further supported by a recent publication from Spain presenting detailed analysis of variation in faecal Hb concentration by lesion characteristic, with adenomas displaying villous features and high grade dysplasia associating with higher median faecal Hb concentration than adenomas with less severe histological features. (Garcia *et al.*, 2015b) The evidence showing that adenomas displaying high-grade dysplasia have a significantly higher median faecal Hb concentration than those with low-grade dysplasia may indicate that the severity dysplastic change can reflect propensity of the lesion to bleed. However, further analysis of this Scottish cohort showed there to be no significant difference between high-grade dysplasia and low-grade dysplasia adenomas when size was taken into account, and retrospective multiple regression modelling demonstrated that adenoma size was the only one of the variables studied found to independently related to faecal Hb concentration. Thus, the relationship

between grade of dysplasia and faecal Hb concentration was found to be primarily related to adenoma size. With adenomas displaying high-grade dysplasia being significantly larger than those with low-grade dysplasia, and with 27.2% of larger adenomas also displaying high-grade dysplasia compared to just 7.5% of smaller lesions, then it can be stated that increasing faecal Hb concentration is associated with larger lesions, which, in turn, are more likely to display more severe dysplasia. It is worth noting that very recent analysis from the Netherlands (van Doorn *et al.*, 2015) reported on multiple linear regression performed on a cohort of 877 participants with a lesion detected following a positive screening test result using a cut-off faecal Hb concentration of 10 µg Hb/g faeces and found that both size and polyp morphology, with faecal Hb concentration highest in polyps with pedunculated shape, to remain significantly associated with faecal Hb concentration ($p < 0.001$ and $p< 0.005$, respectively). The finding that only adenoma size was independently associated with faecal Hb concentration in the cohort from Scotland may be due to the far higher cut-off faecal Hb concentration used, above which small polyps are likely to be largely incidental findings.

Further evidence of a potential continuum of risk with increasing faecal Hb concentration came on investigation of those participants who had colorectal cancer detected following their positive screening result. Although the median faecal Hb concentration of participants with colorectal cancer was not significantly different to those with higher-risk adenoma, 74.4% of those with colorectal cancer had a faecal Hb concentration that was above the upper analytical limit of 200 µg Hb/g faeces, compared to 58.1% of those with higher-risk adenoma as their most serious outcome. However, it should be stated even when looking only at those with faecal Hb concentration > 200 µg Hb/g faeces, the PPV for colorectal cancer was still below 10%, and below 40% for advanced neoplasia. This illustrates the poor specificity of faecal

Hb concentration for the detection of significant colorectal neoplasia. The high number of participants with a false positive result in screening is an important issue in terms of use of resources, unnecessary worry experiences by the patients along with the risks and discomfort associated with colonoscopy.

Within the colorectal cancer group, with the exception of polyp cancers, significant differences in median faecal Hb concentration were not seen between early stage and late stage tumours. However, it is of note that 90.0% of the colorectal cancer cases that were associated with a faecal Hb concentration within the analytical working range (< 200 µg Hb/g faeces), were early stage and that 40.0% were polyp cancers. This compares with 51.7% of colorectal cancer above the limit of the analyser that were early stage. Overall, polyp cancers were significantly smaller than the more invasive cancers and had a significantly lower median faecal Hb concentration, being similar in this regard to adenomas with high-grade dysplasia.

The mean maximum diameter of all malignant tumours was, as expected, significantly greater than all of the various groupings of adenomas studied. The Dukes' C1 rectal cancer with the relatively low faecal Hb concentration of 88.4 µg Hb/g faeces demonstrates the potential anomalies in the relationship between faecal Hb concentration and neoplastic disease stage that may occur. This particular tumour had a maximum diameter that was smaller than the median, with a maximum dimension of 25 mm reported, further giving weight to the argument that it is the size of the lesion rather than its Dukes' stage that is related to the degree of lower gastrointestinal blood loss. This is in keeping with the findings of Levi *et al.* (2007) who found that small proximal cancers had low faecal Hb concentration.

It has been demonstrated that, like gFOBT, FIT is less effective at detecting lesions located in the proximal region of the colon than those found distally. (Haug *et al.*, 2011) 77.8% of adenomas and 69.2% of cancers in this cohort were located in the distal colon or rectum. However, the location of lesions missed by FIT screening is not known and this may simply reflect the true anatomical distribution of colorectal neoplasia. The results of similar investigations by Levi *et al*. (2007) involving subjects from an increased risk population have also shown that median faecal Hb concentration was similar between participants with advanced adenomas detected in the proximal colon and those found distally. Despite distal adenomas in this cohort being significantly larger than those located in the proximal colon, and median faecal Hb concentration appearing to be higher in those with distal adenomas, the findings of this study were in keeping with the observations by Levi *et al*. (2007) in that the difference was not statistically significant ($p = 0.08$).

In addition to previously discussed work which provides mixed conclusions with regard to the relationship between faecal Hb concentration and lesion site, (Ciatto *et al.*, 2007; Levi *et al.*, 2007; van Doorn *et al.*, 2015) a very recent study from Australia has now shown that test positivity with a cut-off faecal Hb concentration of 20 µg Hb/g faeces was significantly more likely in those with distal neoplasia compared with those who had proximal neoplasia detected (odds ratio = 2.10, 95% CI: 1.17 - 3.78), but did not present results of variation in median faecal Hb concentration between the two groups. (Symonds *et al.*, 2015b). However, the finding in the cohort presented in this Chapter that there was no significant difference in median faecal Hb concentration in participants with colorectal cancer detected in different regions of the colon, regardless of the fact that the proximal tumours were significantly larger than those found distally, supports the argument that the success of FIT in screening for colorectal neoplasia depends on its efficacy in detecting Hb from different parts of the colon. (Allison, 2010)

It has been suggested in previous literature that there may be gender differences in test sensitivity with gFOBT, (Brenner *et al.*, 2010) and that a slower colonic transit time in women may be partly responsible for this. If this is the case, it might be expected that proximal colorectal cancer in women would have a lower median faecal Hb concentration than in men, owing to the greater time window for Hb degradation to occur from the site of the lesion to when the faeces is sampled. Unfortunately, the numbers in this cohort are too small to allow conclusions to be drawn from more detailed analysis of variation in faecal Hb concentration according to site and gender, but this is an interesting area for future research.

Some evidence exists that when screening with tests for Hb in faeces, interval cancers may occur more regularly in the rectum than screen-detected colorectal cancer, (Jensen *et al.*, 1992; Steele *et al.*, 2012; Tazi *et al.*, 1999) with the speculated reasons for this finding being faster tumour growth rates (Launoy *et al.*, 1997) and non-haemolysed erythrocytes from rectal tumours not yielding positive FIT or gFOBT. For this reason, median faecal Hb concentration in those with lesions arising in the rectum was analysed separately to that of lesions situated in regions of the colon categorised as proximal or distal. This gives a novel insight compared to other studies that have included the rectum in their definition of the distal colon. However, no significant variation was observed in faecal Hb concentration or lesion size in those with rectal advanced neoplasia compared with elsewhere in the colon. It is of note, however that the late stage cancer with the surprisingly low faecal Hb concentration of 88.4 µg Hb/g faeces was a rectal tumour.

It is also of interest that non-neoplastic pathology, particularly diverticular disease, was not associated with faecal Hb concentration that was significantly different from that

found in those with no pathology was detected. This is an important observation, since it indicates that false positive test resulting from benign disease are likely to be no more common than false positive test results in the absence of any colonoscopic abnormality.

These findings, taken together, may be generally interpreted as follows: faecal Hb concentration can be a predictor of lesion size, with larger lesions more likely to carry features further along the pathway to malignancy, namely high-grade dysplasia and villousness, up to colorectal cancer itself.

This work has important limitations. Firstly, analysis of the distributions of faecal Hb concentration collected was confounded by the upper analytical limit of 200 µg Hb/g faeces. Of the 818 participants who were included in this analysis, 393 had a faecal Hb concentration greater than 200 µg Hb/g faeces. Therefore, an exact quantitative estimate of faecal Hb concentration was not recorded for 48.0% of participants. It is possible that those participants who had large malignant tumours detected had faecal Hb concentration far higher than 200 µg Hb/g faeces, and that median faecal Hb concentration was considerably underestimated in this group. This might explain why there was not a statistically significant difference in median faecal Hb concentration between the colorectal cancer group and the higher-risk adenoma group, despite the malignant lesions being significantly larger.

Another limitation relating to the distribution of faecal Hb concentration is that the conclusions drawn from comparisons made between groups with differing clinical findings come with the caveat that some of the median values had relatively wide 95%

CI. This was evident in some groups with small numbers of participants; for example, those with polyp cancers where there were only six confirmed cases.

Thirdly, since this is observational research from results of a screening evaluation, only participants with a positive result were referred for colonoscopy. This meant that analysis of the relationship between faecal Hb concentration and disease could only be carried out on those with a faecal Hb concentration above the cut-off for test positivity of 80 µg Hb/g faeces. There will, therefore, have been false negative test results for which faecal Hb concentration has not been taken into account when calculating the median values for each group. This issue will, in part, be addressed in the next Chapter of this thesis through analysis of interval cancer identified in this cohort.

It is possible that the results may have been strengthened by the collection of more than one sample from each participant, as advocated in several studies for improving neoplasia detection. (Grazzini *et al.*, 2009; Guittet *et al.*, 2009; Levi *et al.*, 2007; Lieberman & Weiss, 2001; Nakama *et al.*, 1999; Park *et al.*, 2010; Rozen *et al.*, 2010; van Roon *et al.*, 2011) This might have gone some way to eliminate the effect of intermittent bleeding, or bleeding at different rates on different days, causing some lesions to associate with lower than expected faecal Hb concentration. This is possibly demonstrated here by the late stage rectal cancer that gave rise to the relatively low faecal Hb concentration of 88.4 µg Hb/g faeces although, as described earlier, other possibilities are plausible. However, a more recent study by Oort *et al*. (2011) found two-test strategies not to be superior to a single test for the detection of colorectal neoplasia.

These findings from ostensibly healthy, asymptomatic individuals have potential ramifications for future selection of optimum cut-off faecal Hb concentration for test positivity in colorectal cancer screening using FIT, particularly when colonoscopy capacity is limited.

It has been documented that positivity rates are affected by factors such as age and gender, (Moss *et al.*, 2012; Steele *et al.*, 2009; Steele *et al.*, 2010) with both having been shown to have an effect on faecal Hb concentration, (Khalid-de Bakker *et al.*, 2011; McDonald *et al.*, 2012) and studies are emerging with focus on potential use of risk scoring models in colorectal cancer screening programmes. (Aniwan *et al.*, 2015; Auge *et al.*, 2014; Chen *et al.*, 2014; Driver *et al.*, 2007; Kaminski *et al.*, 2014; Omata *et al.*, 2011; Stegeman *et al.*, 2014; Wang *et al.*, 2014; Wong *et al.*, 2014; Yeoh *et al.*, 2011) The findings here could be used to further support the inclusion of faecal Hb concentration in risk scoring systems. With the aim of colorectal cancer screening being to detect colorectal cancer and its precursors, these results reinforce the argument for the use of quantitative FIT with an adjustable cut-off in screening programmes by showing that dysplastic change is more likely to arise in lesions that are larger, and more prone to bleed.

4. The relationship between faecal haemoglobin concentration and interval cancers

4.1 Introduction

Interval cancers are a significant issue in colorectal cancer screening programmes. The Expert Working Group for Right-Sided Lesions and Interval Cancers, World Endoscopy Organization, recently recommended a standardised nomenclature for interval cancer across all colorectal cancer screening modalities and colonoscopy surveillance, providing a definition of interval cancer as: colorectal cancer diagnosed after a screening test or examination in which no cancer is detected and before the date of the next recommended examination. (Sanduleanu *et al.*, 2015)

Minimising the proportion of missed colorectal cancer is crucial to the success of colorectal cancer screening programmes in meeting their primary goal of reducing colorectal cancer mortality. Screening programmes, by nature, only allow measurement of clinical outcomes for those undergoing colonoscopy following a positive screening test result. As a result, it is not possible to directly assess test sensitivity within the screening setting since the prevalence of disease in participants below the cut-off concentration is not known. However, identification of colorectal cancer detected in the interval following a negative test result can allow for calculation

of the proportion of interval cancer; this can act as surrogate measure for test sensitivity and therefore, the effectiveness of the screening programme.

Evidence exists that interval cancers are diagnosed at a later stage and therefore have a worse prognosis than screen-detected colorectal cancer (Tazi *et al*., 1999, Jensen *et al*., 1992). Additionally, Steele *et al.*, (2012) found that although interval cancer had a less favourable stage distribution than screen-detected colorectal cancer, interval cancer had a relatively good prognosis compared to those colorectal cancer cases arising in the group not offered screening over the time period of the three pilot rounds of the Scottish Bowel Screening Programme. This would suggest that despite poor test sensitivity, screening with gFOBT still offers a protective effect even in those with missed colorectal cancer in comparison to no screening.

Although randomised controlled trials (RCT) have shown that guaiac faecal occult blood test (gFOBT) utility in colorectal cancer screening is proven to reduce mortality, (Towler *et al.*, 1998) high proportions of all colorectal cancer diagnosed in the screened population that were interval cancers, here referred to as the interval cancer proportion, are commonly reported. Results from large scale RCT of gFOBT screening in England (Hardcastle *et al.*, 1996) and Denmark (Kronborg *et al.*, 1996) reveal interval cancer proportions of 51.3% and 55.2% respectively. A large non-randomised trial of gFOBT effectiveness conducted in Burgundy calculated the interval cancer proportion higher still at 59.3% (Faivre *et al.*, 1991). Further studies from Denmark (Jensen *et al.*, 1992), Scotland (Steele *et al.*, 2012) and France (Tazi *et al.*, 1999) have also provided evidence that interval cancers consistently account for more than half of colorectal cancer detected in populations screened biennially with gFOBT, indicating poor test sensitivity.

Previously published work has also identified some characteristics more associated with interval cancers than gFOBT screen-detected colorectal cancer. Higher proportions of interval cancer are found in women compared with screen-detected colorectal cancer (Gill *et al.*, 2012; Steele *et al.,* 2012) and significantly more interval cancers have been demonstrated to arise in the right colon than do screen-detected colorectal cancer (Brenner *et al.*, 2012; Cooper *et al.*, 2012; Farrar *et al.*, 2006; Gill *et al.*, 2014; Gill *et al.*, 2012; Hosokawa *et al.*, 2003; Singh *et al.*, 2010; Singh *et al.*, 2006; Steele *et al.*, 2012). Assuming advanced neoplasia was present at the time of the negative gFOBT, these findings suggest that gFOBT may tend to be more likely to detect pathology in men and in the left side of the colon. Furthermore, rectal cancers have been found to be more common amongst interval cancer cases than screen-detected colorectal cancer (Garcia *et al.*, 2015a; Jensen *et al.*, 1992; Steele *et al.*, 2012; Tazi *et al.*, 1999) This may tie in with findings that tumour growth rates vary according to subsite, with Launoy *et al*. (1997) reporting growth to be fastest for rectal cancer . It may be that some interval cancer cases arising in the rectum are more likely to be the result of significant progression towards malignancy of what was perhaps a relatively small precursor lesion at the time of the negative screening test. Another plausible explanation is that the erythrocytes in any blood originating in the rectum have not been haemolysed and the still intact erythrocytes do not yield positive results with either gFOBT or Faecal Immunochemical Tests for haemoglobin (FIT). Interval cancers have also been associated with a worse prognosis, with larger, later stage tumours more frequently reported for interval cancer compared with screen-detected colorectal cancer. (Steele *et al.*, 2012)

With countries worldwide now introducing FIT to replace gFOBT in colorectal cancer

screening programmes due to their various advantages (Fraser, 2011a) and numerous studies demonstrating FIT to be a more sensitive test, particularly for advanced adenoma detection than gFOBT (Rabeneck *et al.*, 2012), it is likely that FIT have the potential, at least over time, to reduce interval cancer proportions. Moreover, quantitative FIT allows programme organisers to select a cut-off faecal haemoglobin (Hb) concentration most appropriate for their programme. However, this poses considerable challenges for countries with limited colonoscopy capacity. To secure a low test positivity rate that matches colonoscopy capacity, high cut-off faecal Hb concentration must be used. This may negate the improved sensitivity offered by FIT over gFOBT; this has been demonstrated by the results of an evaluation of quantitative FIT in Scotland at a cut-off faecal Hb concentration of 80 µg Hb/g faeces in which the Positive Predictive Values (PPV) for advanced neoplasia were no better than with gFOBT. (Steele *et al.*, 2013)

Thus, it is important to establish the interval cancer proportions associated with the use of FIT at a cut-off giving a test positivity rate equivalent to gFOBT and to find out if characteristics such as female gender and location in the proximal colon continue to show positive associations with interval cancer. However, data on interval cancer proportions with population screening with FIT are lacking; these would provide essential insights into how quantitative FIT can be utilised in countries with limited colonoscopy capacity to minimise interval cancer proportions and address the gender inequalities that exist with gFOBT screening. Furthermore, the quantitative nature of modern FIT means that, for the first time, analysis can be performed on faecal Hb concentration of participants at the time of the negative test before diagnosis of interval cancer to assess the value of faecal Hb concentration as a predictor of risk. It would be hoped that this can provide a greater insight into how quantitative FIT can be utilised in countries with limited colonoscopy capacity to minimise interval cancer

proportions and address the gender inequalities that appear to exist with gFOBT screening. For these reasons, the consequences of FIT using a faecal Hb concentration cut-off of 80 µg Hb/g faeces (set to give ca. 2% test positivity rate) were assessed in terms of interval cancer within an established colorectal cancer screening programme. The aims of this analysis were to determine the interval cancer proportion in the group participating in the 'FIT as a First Line Test' evaluation in Scotland to allow comparison with the ca. 50% commonly seen in colorectal cancer screening programmes using gFOBT. Furthermore, it was hoped that the cut-off faecal Hb concentration at which all interval cancers would have been detected could be identified along with the positivity rate that this threshold would have generated.

4.2 Materials and methods

The study cohort was derived from the participants of the 'FIT as a First-Line Test' evaluation, the full process of which was described in the previous Chapter (Chapter 3: The relationship between faecal haemoglobin concentration and severity of colorectal neoplasia).

Linkage with the Scottish Cancer Registry was performed by Information Services Division (ISD) Scotland to identify interval cancer cases (defined as colorectal cancer diagnosed after a negative screening test result with FIT and before the invite to the subsequent screening round after the two year interval used in the Scottish Bowel Screening Programme). Comparison of factors including faecal Hb concentration and gender distribution of colorectal cancer between the group with interval cancer and those with screen-detected colorectal cancer was performed. Interval cancer data

were available for interval cancers diagnosed up to 31 December 2012, meaning that the analysis included only participants with a negative screening result date up to 31 December 2010 and participants with a later result date were excluded from the analysis. The linkage was completed using IBM SPSS statistical software version 21.0 (SPSS Inc., Chicago, IL, USA). Colorectal cancer arising within two years of a negative colonoscopy were referred to as “missed” cancers and not interval cancers.

Deprivation was categorized from individual postcodes using population weighted Scottish Index of Multiple Deprivation (SIMD) 2012 quintiles for analysis of screening outcomes by deprivation. (Information Services Division (ISD) Scotland, 2015b) SIMD identifies small area concentrations of multiple deprivation based on income level, employment, health, education, skills and training, housing, geographical access and crime. The most recent 2012 update has improvements to indicators and methodology from SIMD 2009.

MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software was used for all calculations. The Mann-Whitney U test was used for comparison of median faecal Hb concentration between different groups. Probability of $p < 0.05$ was considered significant. Logistic regression analysis was performed to calculate odds ratio for interval cancer amongst different demographic groups, both unadjusted and adjusted for confounding variables.

4.3 Results

Over the six month screening period for which interval cancer data were available, a total of 30,893 participants in the two NHS Boards responded to screening, with 30,140 participants having a negative test result and 753 having a faecal Hb concentration above the 80 µg Hb/g cut-off faecal Hb concentration for a positive test result. Table 4.1 shows the demographic details of those invited and responding to screening.

104 participants with a positive FIT result did not complete follow-up investigations due to either non-attendance, recently performed colonoscopy, or being deemed unfit for invasive procedures, and were excluded from further analysis. Of 649 participants completing investigations as a result of their positive FIT result, 30 had screen-detected colorectal cancer. 31 cases of interval cancer were identified from follow-up of participants with a negative screening test result to give an interval cancer proportion of 50.8%. Table 4.2 displays characteristics associated with interval cancer and screen-detected colorectal cancer. The numbers of cases of screen-detected colorectal cancer and interval cancer were insufficient to allow for further subgroup analysis by gender.

Table 4.1. Screening outcome of invitees, according to gender, age quintile and Scottish Index of Multiple Deprivation (SIMD) quintile.

	Invited		Responded		Positive test result		Negative test result	
	n	%	n	%	n	%	n	%
Gender								
Men	21,213	48.2	14,459	46.8	432	57.4	14,027	46.5
Women	22,825	51.8	16,434	53.2	321	42.6	16,113	53.5
Age (years)								
50-54	11,491	26.1	7,321	23.7	111	14.7	7,210	23.9
55-59	9,943	22.6	6,820	22.1	158	21.0	6,662	22.1
60-64	9,429	21.4	7,027	22.7	133	17.7	6,894	22.9
65-69	6,389	14.5	4,871	15.8	161	21.4	4,710	15.6
70-74	6,786	15.4	4,854	15.7	190	25.2	4,664	15.5
SIMD quintile								
1 (most deprived)	7,327	16.6	4,386	14.2	146	19.4	4,240	14.1
2	9,428	21.4	6,094	19.7	166	22.0	5,928	19.7
3	7,699	17.5	5,443	17.6	128	17.0	5,315	17.6
4	10,956	24.9	8,127	26.3	186	24.7	7,941	26.3
5 (least deprived)	8,572	19.5	6,809	22.0	127	16.9	6,682	22.2

Table 4.2. Characteristics of interval colorectal cancers and screen-detected colorectal cancer.

	Interval cancers		Screen-detected cancers		p-value
	n	%	n	%	
Total cases	31	50.8	30	49.2	
Gender:					
Men	15	48.4	16	51.6	0.90
Women	16	53.3	14	46.7	
Age quintile (years)*:					
50-54	0	0.0	4	100	0.22
55-59	3	50.0	3	50.0	
60-64	8	66.7	4	33.3	
65-69	5	38.5	8	61.5	
70-74	15	57.7	11	42.3	
Cancer site:**					
Right-sided	13	50.0	13	50.0	0.99
Left-sided	5	50.0	5	50.0	
Rectum	13	52.0	12	48.0	
Dukes' stage:					
A	6	42.9	8	57.1	0.07
B	10	50.0	10	50.0	
C	7	43.8	9	56.3	
D	7	100	0	0.0	
Not known	1	25.0	3	75.0	

* Age at time of invite.

** Right-sided CRC includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.

Table 4.3 shows median faecal Hb concentration and corresponding interquartile range (IQR) at the time of screening in those who were subsequently found to have an interval cancer, allowing comparison of faecal Hb concentration according to gender, colorectal cancer stage, site and time to diagnosis following screening. No statistically significant differences were detected within these categories.

Table 4.3. Median faecal haemoglobin concentration (f-Hb) and interquartile range (IQR) at time of negative screening test in those who had interval cancer.

	n	Median f-Hb (µg Hb/g faeces)	IQR	p-value
All	31	2.8	0.4 - 13.5	
Gender:				
Men	15	12.6	0.1 - 12.9	0.440
Women	16	0.5	0.4 - 11.7	
Stage:				
Total early	16	3.1	0.4 - 12.1	0.466
Total late	14	2.5	0.4 – 6.8	
Site*:				
Right-sided	13	1.4	0.0 – 11.8	0.298
Left-sided	5	15.2	2.3 – 31.1	
Rectum	13	2.8	0.4 – 6.1	
Time to diagnosis:				
within 1 year	8	4.1	0.2 – 7.5	0.786
1-2 years	23	2.8	0.4 – 15.9	

* Right-sided CRC includes region up to and including the splenic flexure; left-sided includes descending and sigmoid colon; rectum includes recto-sigmoid junction and rectum.

46.9% of colorectal cancer cases in men were interval cancers compared with 55.2% in women. Median age in those with an interval cancer was 68 years (95% CI: 64 - 72) compared with 67 years (95% CI: 61 - 71) for screen-detected colorectal cancer cases.

Table 4.4 shows the effect on test positivity rate and interval cancer proportion of lowering the faecal Hb concentration cut-off to various concentrations and Figure 4.1 displays the same data in graphical form. Figure 4.2 shows the number of colonoscopies that would have been required at different faecal Hb concentration cut-offs alongside the associated proportions of interval cancer and screen-detected colorectal cancer. Halving the cut-off faecal Hb concentration to 40 µg Hb/g faeces would have detected 10% more colorectal cancer (assuming lesions would be detected at colonoscopy), reduced the interval cancer proportion from 50.8% to 45.9% but with a significant 58.6% increase in the number of colonoscopies required.

Table 4.4. Effect of lowering the cut-off faecal haemoglobin concentration (f-Hb) on test positivity rate and interval cancer proportion.

Cut-off f-Hb (µg Hb/g faeces)	Positivity rate	Interval cancers		Screen-detected cancers	
		n	%	n	%
80	2.5%	31	50.8	30	49.2
60	3.0%	29	47.5	32	52.5
40	3.9%	28	45.9	33	54.1
20	6.3%	26	42.6	35	57.4
15	7.6%	23	37.7	38	62.3
10	9.5%	23	37.7	38	62.3
2	24.7%	12	19.3	49	80.3
> 0	48.3%	6	9.8	55	90.2

Figure 4.1. Effect of lowering the cut-off faecal haemoglobin concentration (f-Hb) on test positivity rate and interval cancer proportion.

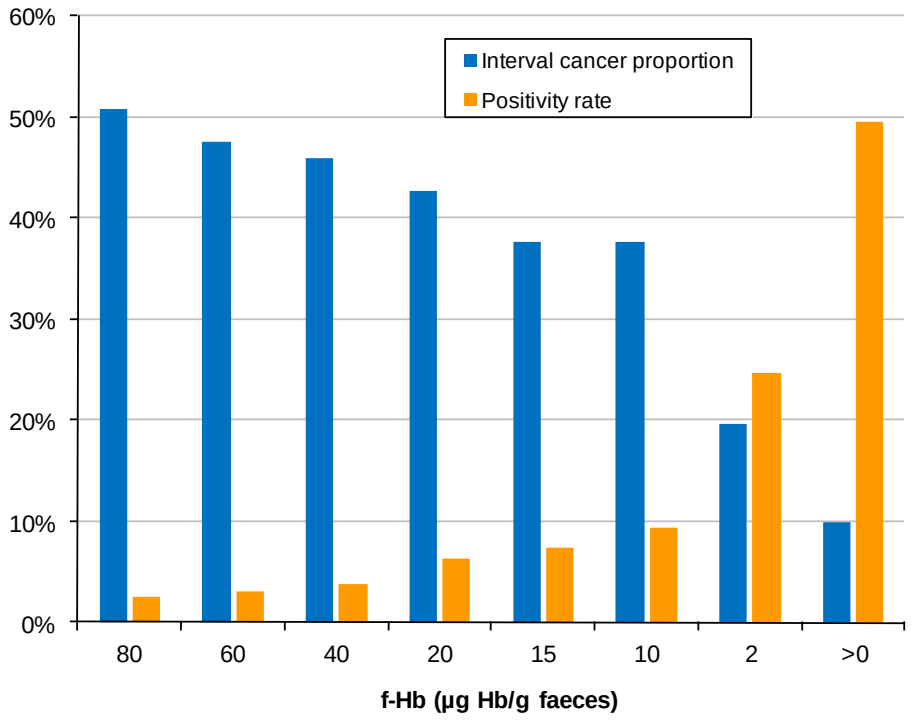


Figure 4.2. Effect of lowering the cut-off faecal haemoglobin (f-Hb) on proportions of interval cancer and screen-detected colorectal cancers (CRC) and number of colonoscopies required.

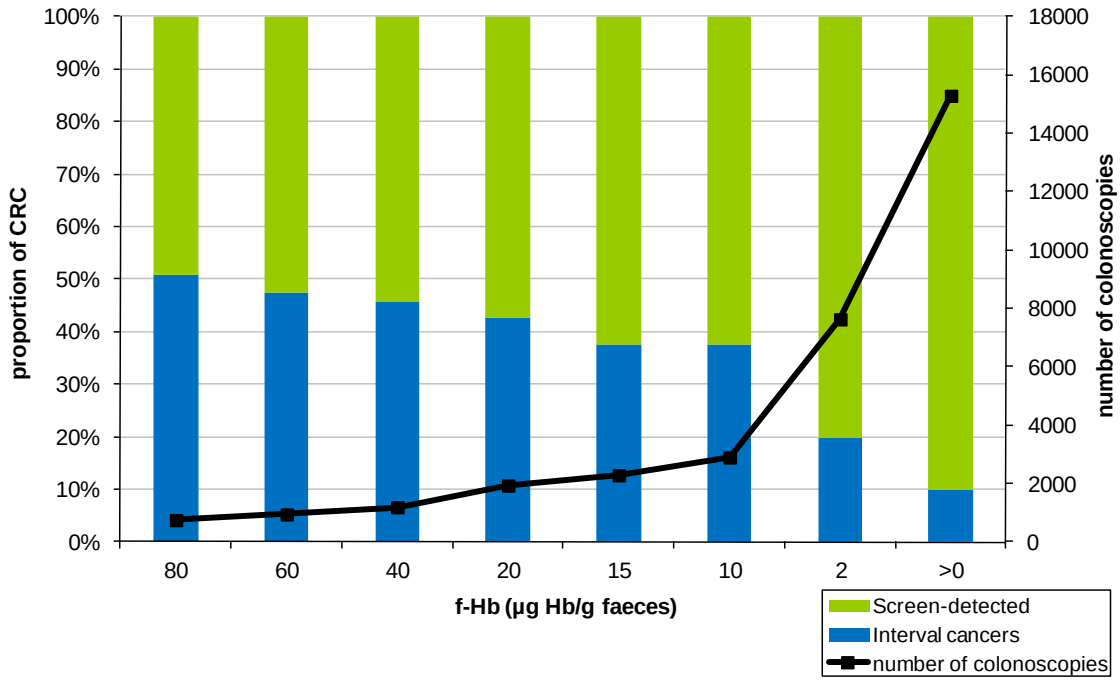
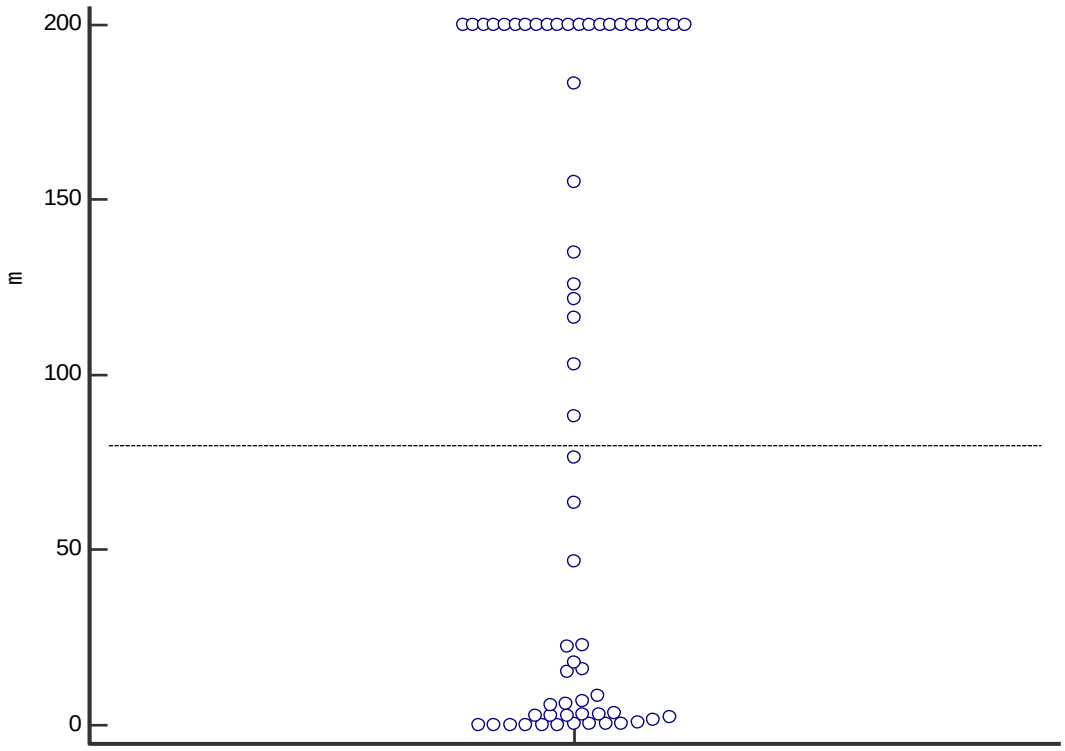


Figure 4.3 shows the faecal Hb concentration distribution of all colorectal cancer cases. Of the 31 interval cancer cases, 23 had faecal Hb concentration less than 10 µg Hb/g faeces at the time of their negative screening test, meaning that over a third of colorectal cancer cases would still have been missed if this cut-off faecal Hb concentration had been adopted. Furthermore, six of these 23 cases had completely undetectable faecal Hb concentration. With 53.0% of all participants having undetectable faecal Hb concentration, the proportion of interval cancers arising in this group was calculated. This group was used as a reference category when producing odds ratios for IC compared with those with higher faecal Hb concentration. Odds ratios were also calculated for men compared with women, and for 60-69 year olds and those over 70 years old compared with those aged 50-59 years. These results along with odds ratio also adjusted for age and gender are displayed in Table 4.5. Figure 4.4 demonstrates the increasing proportion of interval cancers diagnosed at increasing ranges of faecal Hb concentration.

Figure 4.3. Faecal haemoglobin concentration (f-Hb) distribution of all cancers in the screened population.



Dashed line represents cut-off of 80 µg Hb/g faeces

Figure 4.4. Proportion of interval cancers (IC) diagnosed in participants with different ranges of faecal haemoglobin concentration (f-Hb).

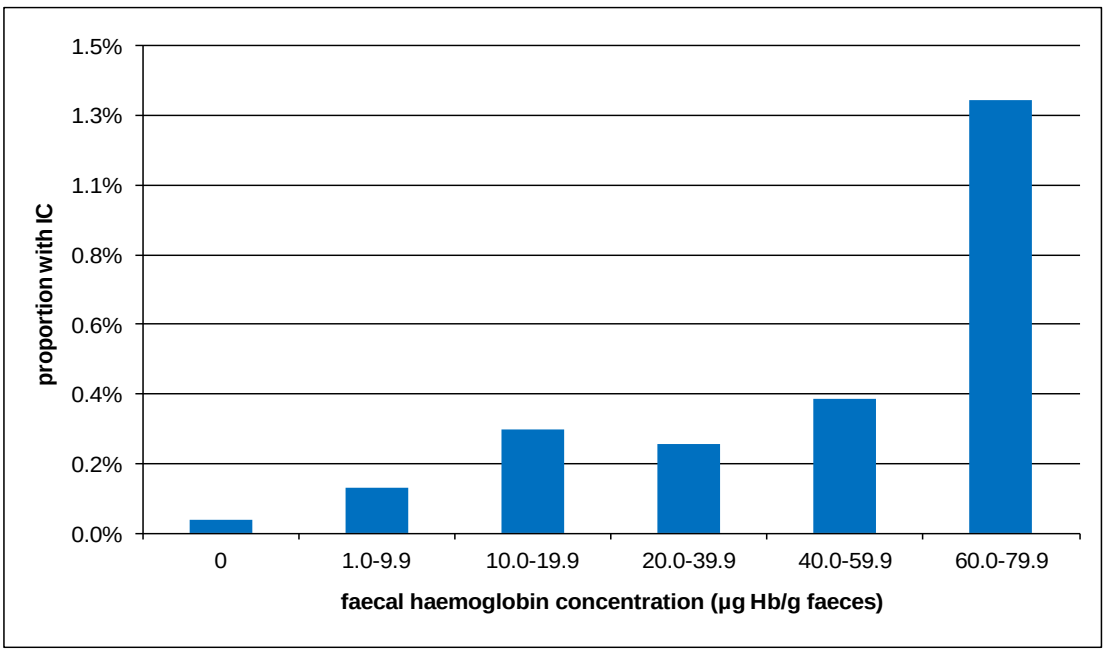


Table 4.5. Proportion of interval cancers by faecal haemoglobin concentration (f-Hb), gender and age with adjusted odds ratios with 95% confidence intervals (CI).

		Odds ratio	
	% with interval cancer	Non-adjusted (95% CI)	Adjusted (95% CI)*
f-Hb (µg Hb/g faeces):			
0.0	0.04	1.00	1.00
1.0 - 9.9	0.17	3.57 (1.41 - 9.06)	3.17 (1.25 - 8.05)
10.0 - 19.9	0.38	8.11 (2.02 - 32.46)	5.95 (1.48 - 24.00)
20.0 - 39.9	0.44	7.03 (1.42 - 34.91)	5.29 (1.06 - 26.40)
40.0 - 59.9	0.75	10.52 (1.26 - 87.73)	8.20 (0.98 - 68.91)
60.0 - 79.9	1.31	34.42 (6.89 - 171.93)	23.91 (4.73 - 120.81)
Gender:			
Women	0.10	1.00	1.00
Men	0.11	1.07 (0.53 - 2.16)	1.01 (0.50 - 2.04)
Age (years):			
50 – 59	0.02	1.00	1.00
60 – 69	0.11	5.15 (1.47 - 18.09)	4.69 (1.33 - 16.47)
≥ 70	0.32	14.61 (4.23 - 50.48)	12.16 (3.50 - 42.26)

*Adjusted for age and gender as applicable.

4.4 Discussion

These results provide unique insights into interval cancer proportions using FIT with a high cut-off faecal Hb concentration in an established screening programme with limited colonoscopy capacity (80 µg Hb/g faeces) and how these rates could be

influenced by varying the cut-off faecal Hb concentration. The interval cancer proportion found was no different to the rates of around 50% commonly reported in literature from screening programmes using traditional gFOBT. It was, however, much higher than the 14.4% interval cancer proportion found in Italy with a cut-off faecal Hb concentration of 20 µg Hb/g faeces. (Zorzi *et al.*, 2011) This indicates that the use of a high cut-off faecal Hb concentration not only limits the improved sensitivity for significant neoplasia offered by FIT but increases the interval cancer proportion. However, assuming these cancers were present and would have been detected at colonoscopy at least in the form of significant precursor lesions at the time of the negative screening test, an interval cancer proportion at a cut-off faecal Hb concentration of 20 µg Hb/g faeces of 42.6% was calculated. Even at this markedly lower cut-off faecal Hb concentration than that used in this evaluation, the interval cancer proportion would still be almost three-times higher than that identified with FIT in Italy. A potential reason for this may be that the region of Italy studied has been offering FIT screening since 2002. With the improved sensitivity for advanced neoplasia demonstrated by FIT compared with gFOBT appearing to be particularly attributable to detection of pre-malignant polyps, (Rabeneck *et al.*, 2012) it may be that removal of such precursor lesions over multiple screening rounds has limited the number of interval cancers in Italy.

It should be mentioned that the calculation of yield of screen-detected colorectal cancer at different cut-off faecal Hb concentration is likely to be a slight underestimation. In addition to avoided interval cancer, a lower cut-off faecal Hb concentration may also have led to detection of colorectal cancer that would arise as screen-detected colorectal cancer at the subsequent screening round as well as a small proportion of over-diagnosed cancers. Therefore, in reality the interval cancer proportion may be lower than reported here, although this is difficult to quantify.

Our results back up previous findings that women have a higher interval cancer proportion than men. In attempting to explain this, characteristics associated with colorectal cancer between the genders were analysed. In contrast to results of previous studies investigating interval cancer in gFOBT screening programmes, this did not reveal an association with location in the proximal colon for interval cancer in women, with just a quarter of cases in women located from the caecum up to and including the splenic flexure. This compared with 38.5% of screen-detected colorectal cancer in women being right-sided. Furthermore, it was in fact the case that most interval cancers in men were right-sided. What was revealed was that over half of cancers (53.3%) diagnosed in women, both interval cancers and screen-detected, were located in the rectum, whereas rectal cancers accounted for a much lower proportion of all colorectal cancer cases in men (29.0%). It can be speculated that this discrepancy in colorectal cancer site distribution between men and women may be contributing towards the inequality in sensitivity of FIT between the sexes. Overall colorectal cancer statistics for Scotland do not echo this pattern of site distribution, with proportions of colorectal cancer located in the rectum or at the recto-sigmoid junction diagnosed in 50-74 year olds in 2011 being 36.8% and 31.2% for men and women, respectively. (Information Services Division (ISD) Scotland, 2015a) Nonetheless, using this cohort, it can be proposed that interval cancer cases in men were mostly attributable to lesions located in the proximal colon that were not bleeding enough to produce a positive FIT result. With rectal colorectal cancer however, the cause for missed lesions may tie in with previously findings of Launoy *et al.* (1997) that progression to malignancy is faster in the rectum than in precursor lesions arising elsewhere in the colon. Smaller precursor lesions that are not bleeding enough at the time of FIT screening to trigger a positive test result may be rapidly becoming malignant before the next screening round. With rectal colorectal cancer much more prevalent in women than men in this cohort, a greater proportion of aggressive rectal neoplasia may offer some explanation for the inequalities seen in the interval cancer

proportion. Incidentally, analysis of adenoma site distribution amongst participants with a positive FIT result attending for colonoscopy shows rectal adenomas to only account for a small proportion of the total adenomas detected in both men and women. However, rectal adenomas were most likely to display villous features and show high-grade dysplasia than adenomas located elsewhere. This is an area requiring further study.

The more advanced stage distribution of interval cancer highlights the need for measures to be taken to improve colorectal cancer detection with screening. Lowering the cut-off faecal Hb concentration would be an obvious solution, but the resultant increase in demand for colonoscopy may not be supported by the available resources. This problem could be counteracted by screening at a low cut-off faecal Hb concentration, but alongside a longer interval between screening rounds than the two years currently implemented. This is an interesting area for future research to investigate the impact of such a strategy on interval cancer proportions.

A concern is that almost 20% of interval cancer cases in this cohort had undetectable faecal Hb concentration meaning that a significant proportion of colorectal cancer would always be missed, even with drastic lowering of the cut-off faecal Hb concentration. Although participants who had undetectable Hb in their sample accounted for over half of the screened population, the proportion of interval cancer in this group was relatively small – over 35 times lower than the proportion of interval cancer identified in those with faecal Hb concentration in the range 60.0 - 79.9 µg Hb/g faeces, who constituted just 0.5% of the cohort. Adjusted odds ratios demonstrate an increasing risk of interval cancer with increasing faecal Hb concentration and perhaps indicate a need for participants with elevated faecal Hb concentration to be offered

more regular screening. This ties in with the work by Chen *et al.* (2011) showing that the higher the initial faecal Hb concentration, the sooner the interval cancer would be found. These results also support the inclusion of faecal Hb concentration in risk scoring models increasingly being documented within colorectal cancer screening populations worldwide.

Another area for future research is better understanding of the potential biological differences between interval cancer and screen-detected colorectal cancer. Recent data from the NHS Bowel Cancer Screening Programme revealed that those with Dukes' stage C and D screen-detected colorectal cancer had superior survival rates in comparison to stage-matched interval cancer ($p < 0.05$). (Gill *et al.*, 2014) The authors stated that the worse prognosis observed for those with an interval cancer, despite an apparently similar degree of disease severity, suggests that some biological difference may exist between interval cancers and screen-detected colorectal cancer. Existing literature has explored association of interval cancer following negative colonoscopy with molecular features such as BRAF and KRAS mutations, chromosomal instability and microsatellite instability, as documented in the 2014 review by Cisyk *et al.*(2014) However, there is a lack of research into the biological features of interval cancers in populations screened with tests for the presence of Hb in faeces that relate to a lesion's propensity to bleed if a false negative, or to undergo rapid growth if a *de novo* colorectal cancer which at the time of screening was a true negative case.

Many other laboratory tests utilise varying reference values partitioned according to factors such as age and gender to reflect the differences in values seen in these demographic groups. Evidence of inequalities in faecal Hb concentration distribution between the genders and with age (Fraser & Auge, 2014; Fraser *et al.*, 2014;

McDonald *et al.*, 2012; Symonds *et al.*, 2015b) raises the question of whether it may be advantageous to adopt a similar strategy in colorectal cancer screening with quantitative FIT. However, men having higher median faecal Hb concentration than women and faecal Hb concentration increasing with age may simply reflect the fact that disease is more prevalent in these groups. Some may be of the opinion that owing to the fact that men have more colorectal neoplasia, a lower cut-off faecal Hb concentration should be used in men than women. Our results would suggest that this approach would widen the inequality seen in interval cancer proportions between men and women. Although it was shown using adjusted odds ratios that overall, women are no more likely than men to have an interval cancer (due to a lower overall percentage of colorectal cancer than in men), women who do have colorectal cancer or advanced precursor lesions are more likely to have faecal Hb concentration below the cut-off and therefore not be referred for colonoscopy to detect and remove the lesion. With interval cancer being associated with worse prognosis, it appears that women may be disadvantaged by the use of one cut-off faecal Hb concentration for all and therefore better individualised use of FIT in colorectal cancer screening should be considered.

5. The relationship between faecal haemoglobin concentration and detection of advanced colorectal neoplasia in the subsequent screening round

5.1 Introduction

It has been established in the previous Chapter that colorectal cancer screening programme participants with elevated faecal haemoglobin (Hb) concentration, although still below the selected cut-off concentration for a positive test result, are more likely to be diagnosed with an interval cancer than those with undetectable haemoglobin (Hb) in their screening sample. Further knowledge of the predictive power of faecal Hb concentration in colorectal cancer screening may be obtained from retrospective analysis of faecal Hb concentration in participants who have had neoplasia detected following a positive screening test result in the round subsequent to a negative test result. Such analysis could provide evidence to support future modification of protocol to improve the effectiveness of the screening programme. For example, those at elevated risk of having advanced neoplasia, despite their negative screening test result, could be prioritised for future repeat screening at a shorter interval than the current biennial invitation. Conversely, those deemed very unlikely to have a later diagnosis of advanced neoplasia might not require such frequent invitation to screening. Since it has already been established that those with undetectable faecal Hb concentration account for around half of all participants in this large cohort completing a single Faecal Immunochemical Test for Hb (FIT) as a first-line test, this

strategy could potentially significantly reduce the number of overall referrals to colonoscopy. This, in turn, could pave the way for lowering of the cut-off faecal Hb concentration to improve test sensitivity and therefore reduce the interval cancer proportion. This is a particularly important consideration in a country such as Scotland where it has been necessary to adopt a cut-off faecal Hb concentration that is significantly higher than that used elsewhere, due to the limited capacity of the colonoscopy resource.

The existing literature provides evidence that a trend exists between increasing faecal Hb concentration and more severe colorectal findings being detected at follow-up to a positive screening test result, as reviewed in Chapter 3 (Faecal haemoglobin concentration is related to severity of colorectal neoplasia), and further supported by the new findings reported in the results that followed. What has been more rarely documented, however, is the relationship between faecal Hb concentration that is below the cut-off and treated as a baseline measurement, and clinical outcomes in the longer term to determine the role of faecal Hb concentration as a predictor of future risk of advanced neoplasia. One important study that does investigate this is the longitudinal follow-up of colorectal cancer screening participants in Taiwan by Chen *et al.* (2011) The authors followed-up a cohort of 44,324 participants aged 40 - 69 years with faecal Hb concentration below the cut-off concentration of 20 µg Hb/g faeces) for a median of 4.39 years (interquartile range 2.53 - 6.12). Incidence rates and hazard ratios for advanced neoplasia in those with baseline faecal Hb concentration within incremental ranges up to the cut-off faecal Hb concentration were calculated. The incidence of advanced neoplasia rose from 1.75/1000 person-years for those with faecal Hb concentration 0.2 – 3.9 µg Hb/g faeces to 7.08/1000 person-years in those closest to the cut-off concentration with faecal Hb concentration between 16.0 and 19.9 µg Hb/g faeces. Moreover, relative to those with faecal Hb concentration 0.2 – 3.9 µg

Hb/g faeces, adjusted hazard ratios for advanced neoplasia were calculated as 3.41 (95% confidence interval [CI] 2.02 - 5.75) for those with faecal Hb concentration in the category closest to the cut-off faecal Hb concentration used. This trend indicates that the higher the baseline faecal Hb concentration, the greater the likelihood of a future diagnosis of advanced neoplasia. Another interesting finding was that analysis of participants for whom faecal Hb concentration was measured in subsequent screening rounds following the baseline faecal Hb concentration showed that the group who were eventually diagnosed with colorectal neoplasia displayed a trend of rising median faecal Hb concentration across three screening rounds. This was in contrast to a less pronounced general trend of decreasing faecal Hb concentration in the second and third rounds in those with no diagnosis of colorectal neoplasia. From these data arises the interesting concept of monitoring change in participant's found faecal Hb concentration across screening rounds to identify those at greater risk. Implementation of such a strategy in practice may be complicated, but the evidence from this novel study yet again highlights the numerous opportunities for more efficient and effective screening facilitated by the replacement of guaiac faecal occult blood tests (gFOBT) with FIT.

A recent update of this group's work is given by the paper published by Yen *et al*., (2014) where results of their regression modelling work are presented to further establish the value of faecal Hb concentration as a predictor for colorectal neoplasia. 54,921 participants invited between 2001 and 2007 were followed up in the annual FIT screening programme to identify those diagnosed with interval cancer, screen-detected colorectal cancer and adenoma. In addition to follow-up of participants with a negative FIT result at baseline, participants with an initial faecal Hb concentration above the cut-off concentration of 20 µg Hb/g faeces were also included in the model. As with the study by Chen *et al*., (2011) a trend of increasing hazard ratios for colorectal neoplasia

with increasing baseline faecal Hb concentration was demonstrated, but this time extended well above the selected cut-off faecal Hb concentration with the inclusion of these extra data. The area under the curve for the model to assess the risk of colorectal cancer rose from 0.67 (95% CI: 0.64 - 0.70) when only including conventional risk factors such as gender, family history, smoking and Body Mass Index, to 0.84 (95% CI: 0.82 - 0.87) when faecal Hb concentration was incorporated into the model, with the relationship stronger in men than women.

Literature around the topic of the value of faecal Hb concentration as a predictor of future risk is scarce, with only two studies identified, both concerning the same Taiwanese population. It is accepted that due to variation in available resources and in the distribution of faecal Hb concentration across geography, countries using FIT as the initial screening investigation must conduct their own evaluation into the predictive value of faecal Hb concentration for future diagnosis of advanced neoplasia, if seeking to incorporate into their screening programmes more creative strategies to improve detection rates and programme efficiency. With the aim of not exceeding the capacity of the available colonoscopy resource, organisers of the Scottish Bowel Screening Programme selected a cut-off faecal Hb concentration for the evaluation of using FIT as a first-line test that was far higher than that used elsewhere, meaning that many participants with a test result below the cut-off faecal Hb concentration would have been deemed to have a positive test result and followed up with colonoscopy if screened in these other countries. As a result, it can be expected that a reasonable proportion of participants with faecal Hb concentration below the cut-off concentration in the Scottish FIT evaluation cohort would have advanced neoplasia diagnosed at the subsequent screening round.

The aim of this section was to investigate the relationship between faecal Hb concentration at the time of the negative screening test result during the FIT evaluation and the screening test result at the subsequent screening round. It was also hoped to determine ifthose with faecal Hb concentration closest to the cut-off were more likely to be subsequently diagnosed with advanced neoplasia. If this is the case, further evidence of the predictive power of faecal Hb concentration in colorectal cancer screening will be provided and consideration of closer surveillance of participants with elevated faecal Hb concentration, although below the cut-off concentration, may be warranted.

5.2 Materials and methods

The study cohort was again derived from the participants of the 'FIT as a First-Line Test' evaluation, the full process of which was previously described in the previous Chapter (Chapter 3: The relationship between faecal haemoglobin concentration and severity of colorectal neoplasia).

Following completion of the 'FIT as a First-Line Test' evaluation, the Scottish Bowel Screening Programme returned to the two-tier reflex gFOBT/FIT screening algorithm, which has been described in Chapter 2: The relationship between results with the gFOBT/FIT two-tier reflex screening algorithm and severity of colorectal neoplasia.

The screening test results for all of those eligible to take part in the subsequent screening round after the FIT evaluation and resident in either NHS Tayside or NHS Ayrshire & Arran were examined. Data for colonoscopy outcomes and any subsequent

pathology for those with a positive gFOBT/FIT screening test result were downloaded and collated as described previously in this work.

MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software was used for all calculations in this analysis. The Mann-Whitney U test was used for comparison of median faecal Hb concentration between groups. Probability of $p < 0.05$ was considered significant. Logistic regression analysis was performed to calculate odds ratios for diagnosis of advanced neoplasia at the next screening round amongst those in different faecal Hb concentration categories and different demographic groups, both unadjusted and adjusted for age and gender as known confounding variables.

5.3 Results

37,780 participants had faecal Hb concentration below the cut-off used of 80 µg Hb/g faeces. 92.7% were invited for screening with the gFOBT algorithm in the next round; the majority of those not invited were above the age range of the screening programme, with others having died or no longer being resident in Scotland. 30,849 participants completed both the FIT evaluation and the subsequent screening round when the programme had reverted to the gFOBT/FIT two-tier reflex algorithm. Table 5.1 details the screening outcomes of all participants who had faecal Hb concentration below the 80 µg Hb/g faeces cut-off used in the FIT evaluation, by age and gender.

Table 5.1. Subsequent screening result of participants with negative test result.

	Total		Men		Women	
	n	%	n	%	N	%
Total with faecal haemoglobin concentration <80 µg Hb/g faeces	37,780		17,525		20,255	
Result in subsequent round:						
Positive test	556	1.5	339	1.9	217	1.1
Negative test	30,293	80.2	13,910	79.4	16,383	80.9
Non-responder	4,165	11.0	1,976	11.3	2,189	1.8
Excluded	2,766	7.3	1,300	7.4	1,466	7.2

A total of 556 (1.5%) participants went on to have a positive test result in the subsequent screening round, and 30,293 (80.2%) were again deemed to have a negative screening test result. The median faecal Hb concentration was statistically significantly higher in those who had a positive test result in the subsequent round than those who again had a negative test result (2.1 µg Hb/g faeces, IQR 0.0 - 13.2 v. 0.0 µg Hb/g faeces, IQR 0.0 - 1.4; $p < 0.0001$).

Table 5.2 displays the clinical outcomes with median faecal Hb concentration of participants who had a positive result in the subsequent screening round.

The majority, 96.6%, of participants of both rounds had an initial faecal Hb concentration in the lowest category, that is 0.0 - 19.9 µg Hb/g faeces. Of those

undergoing follow-up to a positive test result in the subsequent screening round, 87.4% of participants who did not have advanced neoplasia detected were in this low faecal Hb concentration group, compared to only 56.8% of those with advanced neoplasia having had their previous faecal Hb concentration in this lowest category examined. The proportion of participants who had faecal Hb concentration within different categories of faecal Hb concentration, by their final outcome at the subsequent screening round, are shown in Table 5.3.

Table 5.2. Clinical outcomes and median baseline faecal haemoglobin concentration (f-Hb) with interquartile range (IQR) in participants with a positive screening test result in the subsequent screening round.

	All				Men				Women			
	n	%	median f-Hb (µg Hb/g faeces)	IQR	n	%	median f-Hb (µg Hb/g faeces)	IQR	n	%	median f-Hb (µg Hb/g faeces)	IQR
Total with a positive screening test result in the subsequent screening round	556		2.1	0.0 - 13.0	339		1.6	1.2 - 3.0	217		2.4	0.0 - 12.9
Cancer (CRC)	26	4.7	16.7	1.2 - 31.6	19	5.6	21	0.5 - 36.3	7	3.2	10.2	2.5 - 24.0
Higher-risk adenoma (HRA)	85	15.3	13.6	1.2 - 38.5	63	18.6	9.6	0.9 - 38.8	22	10.1	16.6	1.8 - 35.2
Advanced neoplasia (CRC + HRA)	111	20.0	13.6	1.2 - 37.6	82	24.2	14.7	0.8 - 38.8	29	13.4	13.6	1.8 - 35.2
Low-risk adenoma	65	11.7	1.7	0.0 - 8.5	42	12.4	1.6	0.0 - 10.9	23	10.6	1.8	0.0 - 7.0
Non-neoplastic pathology*	131	23.6	1.6	0.0 - 7.4	72	21.2	0.7	0.0 - 5.8	59	27.2	3.4	0.1 - 15.1
No pathology detected	169	30.4	1.4	0.0 - 6.2	93	27.4	0.3	0.0 - 5.6	76	35.0	2.0	0.0 - 7.3

* - Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

Table 5.3. Proportion of participants in each faecal haemoglobin (Hb) concentration category, by final outcome.

	Faecal Hb concentration (µg Hb/g faeces)							
	0.0 - 19.9		20.0 - 39.9		40.0 - 59.9		60.0 - 79.9	
	n	%	n	%	n	%	n	%
Negative screening test result	29,049	96.6	661	2.2	227	0.8	125	0.4
Positive screening test result	449	80.8	59	10.6	26	4.7	22	4.0
Cancer (CRC)	14	53.8	7	26.9	1	3.8	4	15.4
Higher-risk adenoma (HRA)	49	57.6	17	20.0	10	11.8	9	10.6
Total advanced neoplasia (CRC + HRA)	63	56.8	24	21.6	11	9.9	13	11.7
Low-risk adenoma	58	85.3	8	11.8	2	2.9	0	0.0
Non-neoplastic pathology*	115	87.8	6	4.6	7	5.3	3	2.3
No pathology detected	147	88.0	14	8.4	2	1.2	4	2.4

* - Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

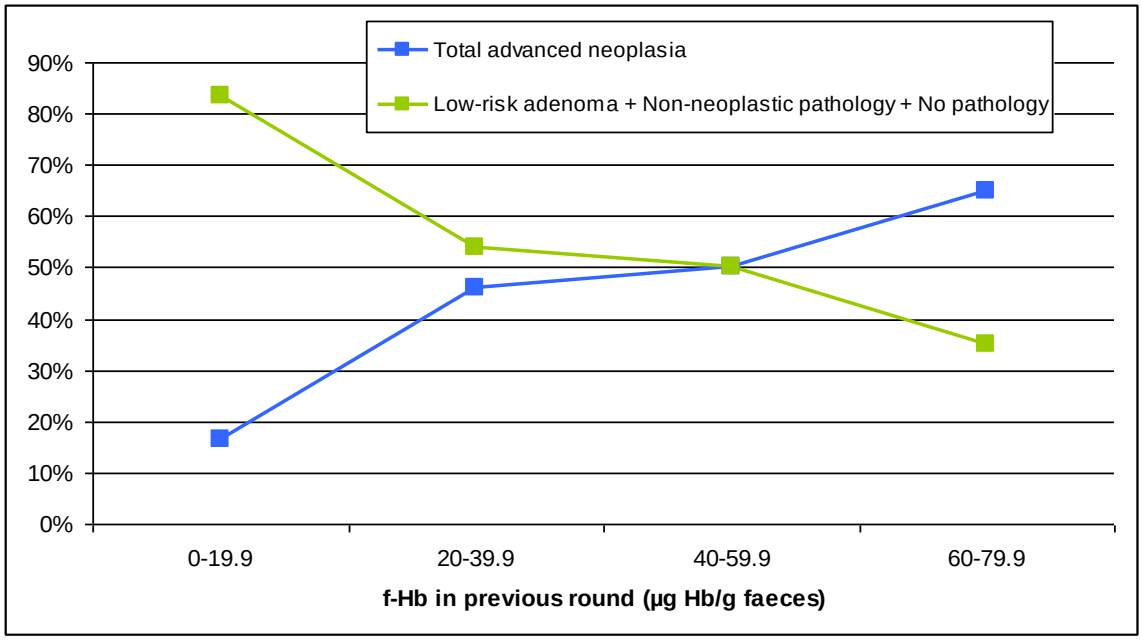
Table 5.4 shows the proportion of participants with different clinical outcomes according to which category of faecal Hb concentration they fell into at the previous screening round with FIT. The majority of participants with a positive screening test result who had previously low concentrations of faecal Hb concentration (< 60 µg Hb/g faeces) did not have advanced neoplasia detected at the next round. However, the opposite was true with rising faecal Hb concentration. This can be visualised in Figure 5.1. Figure 5.2 shows the percentage of participants in each faecal Hb concentration category with or without advanced neoplasia detected at the subsequent screening round, this time presented as a proportion of all participants of both rounds.

Table 5.4. Clinical outcomes expressed as the proportion of participants with follow-up complete after a positive screening result in the subsequent round, by faecal haemoglobin concentration (faecal Hb concentration) category.

	Faecal haemoglobin concentration (µg Hb/g faeces)							
	0.0 - 19.9		20.0 - 39.9		40.0 - 59.9		60.0 - 79.9	
	n	%	n	%	n	%	n	%
Follow-up complete	383		52		22		20	
Cancer (CRC)	14	3.7	7	13.5	1	4.5	4	20.0
Higher-risk adenoma (HRA)	49	12.8	17	32.7	10	45.5	9	45.0
Advanced neoplasia (CRC + HRA)	63	16.4	24	46.2	11	50.0	13	65.0
Low-risk adenoma	58	15.1	8	15.4	2	9.1	0	0.0
Non-neoplastic pathology*	115	30.0	6	11.5	7	31.8	3	15.0
No pathology detected	147	38.4	14	26.9	2	9.1	4	20.0

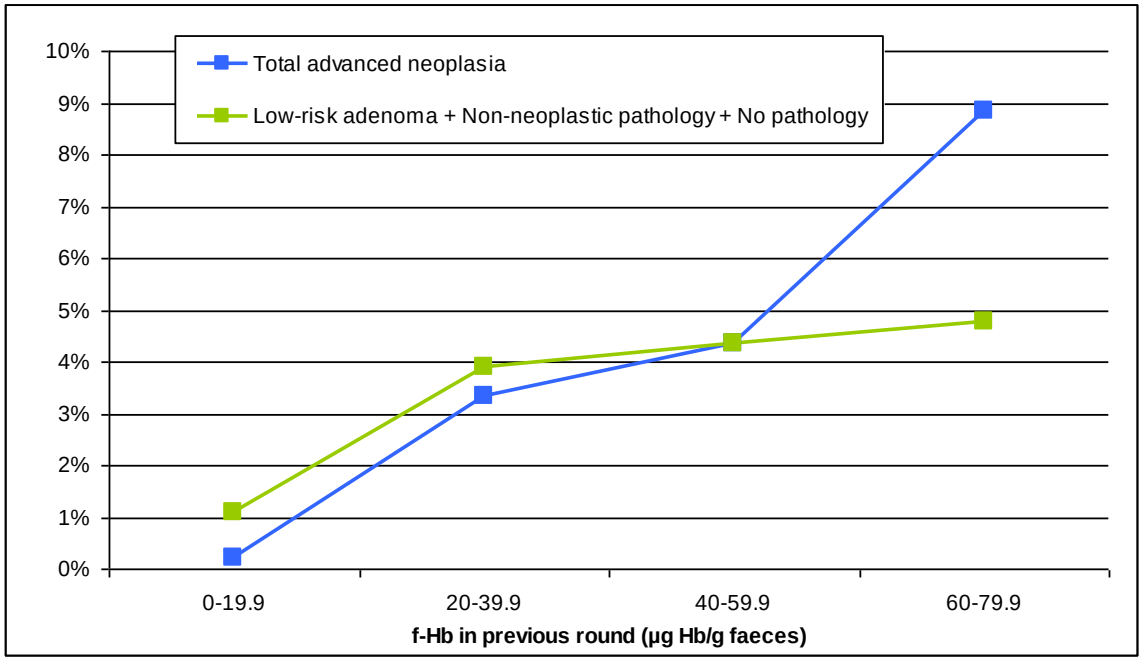
* - Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

Figure 5.1. Proportion of participants with a positive screening test result completing follow-up who had advanced neoplasia or less severe outcomes, according to previous faecal haemoglobin concentration (f-Hb) category.



LRA = low-risk adenoma; Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

Figure 5.2. Proportion of participants of both rounds who had advanced neoplasia or less severe outcomes, according to previous faecal haemoglobin concentration (f-Hb) category.



LRA = low-risk adenoma; Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

The proportion of all participants of both rounds who had advanced neoplasia detected following a positive test result rose with each increasing category of faecal Hb concentration at the previous round. Logistic regression analysis, summarised in Table 5.5, showed very high adjusted odds ratios for advanced neoplasia even in those with faecal Hb concentration 20.0 - 39.9 µg Hb/g faeces, using those with faecal Hb concentration 0.0 - 19.9 µg Hb/g faeces as the reference group (adjusted odds ratio = 14.3, 95% CI: 8.9 - 23.1). Almost 9% of all participants who had faecal Hb concentration within the highest faecal Hb concentration range examined, 60.0 - 79.9 µg/g faeces, and participated in the subsequent round, had advanced neoplasia detected at the follow-up investigations of a positive test result. Advanced neoplasia was over 40-times more prevalent in this group than those with faecal Hb concentration

previously 0.0 - 19.9 µg/g faeces, of whom 0.21% had advanced neoplasia. The adjusted odds ratio for advanced neoplasia in the highest range of faecal Hb concentration was calculated to be 38.0 (95% CI: 20.2 - 71.2).

Table 5.5. Odds ratios (both unadjusted and adjusted for age and gender) with 95% confidence interval (CI) for advanced neoplasia (AN) according to faecal haemoglobin concentration (f-Hb) category.

		Odds ratio	
f-Hb (µg Hb/g faeces)	% with AN detected	Non-adjusted (95% CI)	Adjusted (95% CI)
0.0 - 19.9	0.21	1.0	1.0
20.0 - 39.9	3.37	16.2 (10.1 - 26.2)	14.3 (8.9 - 23.1)
40.0 - 59.9	4.42	21.5 (11.2 - 41.4)	17.7 (9.2 - 34.2)
60.0 - 79.9	8.97	45.9 (24.7 - 85.4)	38.0 (20.2 - 71.2)

5.4 Discussion

With studies in this area scarce, these results have important implications for future screening strategy. The finding that a far higher proportion of participants with a previous faecal Hb concentration approaching the cut-off used had advanced neoplasia detected at the next round provides further evidence that faecal Hb concentration is a strong predictor of future risk.

The test positivity rate in those participants who, in the previous round, had faecal Hb concentration below the cut-off faecal Hb concentration for positivity of 80 µg/g faeces

was 1.5%. This was lower than the overall test positivity rate seen in the programme with the gFOBT/FIT two-tier reflex algorithm of around 2%. (Steele *et al.*, 2009) This suggests that even with a relatively high cut-off faecal Hb concentration, those who have previously had a negative screening test result are less likely to have a positive test result in the next screening round. This is compared with the overall population invited for screening, which would include first-time participants and repeat participants who have previously had a positive test result. This finding supports very recently published work from Spain showing that test positivity rate in the second round was significantly lower than in the first round, using a cut-off faecal Hb concentration of 20 µg Hb/g faeces (4.8% v. 6.9%, respectively, $p < 0.0005$). (Bujanda *et al.*, 2015)

Despite a lower risk of having a positive test result than the general screening population, it is without doubt that a greater risk of advanced neoplasia is seen in those who had faecal Hb concentration approaching the cut-off used in the previous round. Faecal Hb concentration was significantly elevated, not only in those who would go on to have a positive screening test result in the next round compared to those who again had a negative test result, but also in those who had advanced neoplasia detected at follow-up investigations compared with those with no pathology (both $p < 0.0001$). These findings further enhance the status of faecal Hb concentration as a valuable predictor of risk of advanced neoplasia. Furthermore, one in five participants who had faecal Hb concentration in the category closest to the cut-off, then had a positive test result in the subsequent screening round, were diagnosed with colorectal cancer. This compares with one in 27 participants in the lowest faecal Hb concentration category examined, meaning those with an elevated faecal Hb concentration who go on to have a positive test screening result in the next round are over five times more likely to have colorectal cancer detected. When also taking into account detection of higher-risk adenoma, 65% of those participants with a positive screening test result who previously

had faecal Hb concentration in the highest category examined had advanced neoplasia diagnosed, compared to just 16.4% in the lowest category. Moreover, a trend exists of an increasing proportion of advanced neoplasia diagnosed in participants from each of the four increasing ranges of faecal Hb concentration. From these data, the odds of having advanced neoplasia detected following a positive screening test result are even in those with previous faecal Hb concentration between 40.0 and 59.9 μg/g faeces. After this point, it is more likely that not that advanced neoplasia will be present at follow-up investigations in those with a positive screening test result in the subsequent round. When taking into account not just those with a subsequent positive screening test result, but all participants of both screening rounds examined in this analysis, it was seen that almost 1 in 10 of all participants with faecal Hb concentration approaching the cut-off used went on to have advanced neoplasia detected, representing a 40-fold increase in the risk in those in the lowest faecal Hb concentration category. This finding is important when considering faecal Hb concentration as a predictor of future risk of advanced neoplasia at the time of a negative screening test result. Not only is strong evidence provided for the value of faecal Hb concentration as a risk factor for advanced neoplasia being detected at follow-up investigations of participants going on to have a positive test result, but also for predicting future test positivity and the subsequent detection of advanced neoplasia.

There are different ways in which these results can impact on screening strategy. Risk scoring models are becoming increasingly developed for use in colorectal cancer screening programmes, with an escalation in published studies in recent years. (Aniwan *et al.*, 2015; Auge *et al.*, 2014; Chen *et al.*, 2014; Stegeman *et al.*, 2014; Wang *et al.*, 2014; Wong *et al.*, 2014; Kaminski *et al.*, 2014; Chong *et al.*, 2013; Omata *et al.*, 2011; Yeoh *et al.*, 2011; Driver *et al.*, 2007) The results in this Chapter indicate that programmes using FIT and looking to utilise such systems should certainly incorporate

faecal Hb concentration into the models to improve their predictive power. Further potential for application of this finding into screening lies in the idea of using faecal Hb concentration as a determinant of the length of time until the next screening invite. Participants with a negative screening test result, but with faecal Hb concentration close to the cut-off used, could be recalled for screening at a shorter interval than those with very low faecal Hb concentration, whose next invite could be delayed, backed by the evidence that their risk of advanced neoplasia is substantially reduced. Such prioritisation of screening participants according to risk has the potential to improve the performance of screening programmes by targeting resources more effectively.

It can be deduced from the results that more individuals with false positive test results occur at the subsequent screening round in those whose previous faecal Hb concentration was low. This may suggest that the bleeding that producing the positive test result is more likely to be the result of a short term cause such as infection, or haemorrhoids, for example. On the other hand, those with a positive test result, who have previously exhibited an elevated concentration of Hb in the faeces are more likely to have disease. Consideration may be given to targeting the colonoscopy resource towards such participants who have exhibited high faecal Hb concentration in consecutive screening rounds and are, according to the findings of this study, far more likely to have advanced neoplasia. However, such a strategy would come with the caveat that, as documented in the previous Chapter, a proportion of interval cancers are associated with undetectable faecal Hb concentration.

An interesting observation, worthy of further discussion, is that median faecal Hb concentration of participants with colorectal cancer detected at the next screening round was higher in men than in women, but the opposite was apparent for higher-risk

adenoma. In fact, previous median faecal Hb concentration for women with colorectal cancer was very similar to previous median faecal Hb concentration of men with higher-risk adenoma. This may further demonstrate the variation in faecal Hb concentration by gender as now documented using data from various countries, (Fraser & Auge, 2014; Fraser *et al.*, 2014; McDonald *et al.*, 2012; Symonds *et al.*, 2015b) and also by the results discussed in previous Chapters showing that women with advanced neoplasia had lower median faecal Hb concentration than men. A number of theories can be hypothesised here, including gender differences in site distribution, lesion size, colonic transit time and tumour growth rates. Data on site distribution and lesion size at the time following the positive test result in the subsequent round are available, but with only 19 cases of colorectal cancer in men and seven in women, the sample size is not sufficient to draw robust conclusions. It would be interesting to further study why median faecal Hb concentration at the previous round for women with colorectal cancer equates to that of men with the less severe diagnosis of higher-risk adenoma.

An obvious weakness of this analysis is that different screening tests were employed in the two rounds examined. As a result, the findings should be interpreted with the caveat that the cohort of participants with a positive test result at the subsequent screening round may have been different if FIT with a cut off of 80 µg Hb/g faeces was the initial screening test in both rounds. It would be of interest to conduct a similar study where quantitative FIT was used consecutively to allow the variation in faecal Hb concentration over time, according to clinical outcomes to be investigated. Chen *et al*. (2011) documented how, in their cohort, those who had an eventual diagnosis of colorectal neoplasia had a trend of increasing mean faecal Hb concentration over time, whereas the mean faecal Hb concentration of those without neoplasia showed a slight overall decrease across screening rounds. It would have been interesting to test if this

result is echoed in the Scottish population; published data on intra-participant faecal Hb concentration variability are limited and may have implications for future individualised risk assessment using FIT. In addition, the number of colorectal cancer cases is low, limiting detailed analysis by sub-groups such as age, gender and lesion site for example.

Previous Chapters have discussed the relationship between faecal Hb concentration and severity of colorectal disease at immediate follow-up investigations, and also the risk of diagnosis of interval cancer. With the addition of this work, further support has been provided for the case of using faecal Hb concentration as a risk factor, not only at the time of screening, but now also as a longer term predictor of risk. Individuals identified as being at greater risk, although having a screening test result that is negative according to the selected cut-off faecal Hb concentration, could receive their repeat invite for screening at a shorter interval than those who consistently return samples with undetectable faecal Hb concentration, for example. In addition, providing screening participants who have elevated faecal Hb concentration with the knowledge that they are at greater risk of future diagnosis of advanced neoplasia may be beneficial to adherence to subsequent screening invites and awareness of any symptoms arising between invites.

6. The relationship between faecal haemoglobin concentration and degree of deprivation

6.1 Introduction

There is a well-established relationship between deprivation and cancer mortality. Using guaiac faecal occult blood tests (gFOBT) in screening programmes, considerable evidence accumulated that deprivation as well as male sex and older age is associated with an increased incidence of colorectal cancer. (Mansouri *et al.*, 2013; Moss *et al.*, 2012; Steele *et al.*, 2010) The most recent whole-population colorectal cancer statistics for Scotland show a slight trend of rising colorectal cancer incidence with increasing degree of deprivation, while colorectal cancer mortality shows a more marked association (Information Services Division (ISD) Scotland, 2014). Where previous UK evidence had revealed affluence to show association with colorectal cancer incidence in both sexes in the 1980s, this trend faded into the 1990s before beginning to demonstrate a reversal of this relationship. (Oliphant *et al.*, 2011) Age-standardised incidence rates prior to commencement of the Scottish Bowel Screening Programme in the West of Scotland revealed an effect of increasing colorectal cancer incidence with increasing degree of deprivation only in men, cited as being attributable to a fall in colorectal cancer incidence in men in affluent groups rather than a rise in incidence rates in the more deprived. More convincing evidence of an association between increasing deprivation and higher rates of colorectal cancer incidence, independent of other risk factors, comes from the US. (Doubeni *et al.*, 2012)

Interestingly, in this instance, the association was strongest for cancer arising in the rectum and weakest for right-sided colorectal cancer, perhaps indicating the involvement of a particular biological factor driving the relationship. The exact factors causing the associations generally seen between deprivation and colorectal cancer incidence and mortality are unclear, but are commonly suggested to include varying exposures to modifiable lifestyle-related risk-factors, as well as inequalities in access to colonoscopies and screening. Cancer preventability estimates are available for the UK according to various lifestyle-related factors including consumption of red meat, processed meat, alcohol intake, physical activity and body fatness, contributing to a total of 47% of colorectal cancer considered as preventable through eating healthily, being physically active and maintaining a healthy weight (World Cancer Research Fund International (WCRF), 2014). Another study has this figure as high as 54.4%, with the effect greater in men, and meat consumption accounting for the largest proportion of preventable colorectal cancer cases overall of all lifestyle-related exposures examined, at 21.1% (Parkin *et al.*, 2011). With less desirable lifestyle choices being associated with those with higher degree of deprivation in Scotland (Bromley *et al.*, 2010), it follows that more preventable cancers may be occurring in the most deprived. Moreover, Ellis *et al.* (2012) calculated that 11% of overall cancer-related deaths a year in England between 2004 and 2006 would have been avoided if three-year survival had been as high for all patients as in the most affluent groups . It is also widely-known that screening participation is poorer in the most deprived (Steele *et al.*, 2013) and it can be projected that this disparity will widen any pre-existing elevated colorectal cancer risk and deficits in survival rates in this group.

It is important to consider in detail how the introduction of screening programmes may affect the observed relationship between deprivation in colorectal cancer incidence and mortality. Studies have shown degree of deprivation to impact on various stages

throughout the colorectal cancer screening process. In Scotland, increasing deprivation is associated with lower uptake, higher test positivity rates, poorer attendance at follow-up colonoscopy and lower Positive Predictive Values (PPV) for neoplasia. Following multivariate analysis, the relationship between increasing deprivation and lower PPV for detection of neoplasia has been shown to remain only in males. Moreover, deprivation impacted on a number of outcomes in those with colorectal cancer with more deprived groups demonstrating poorer cancer specific and overall survival (Mansouri *et al.*, 2013).

Dietary factors are often cited as contributing towards some of the individuals with false positive test results in colorectal cancer screening with guaiac faecal occult blood test (gFOBT), with detection of haem from red meat consumption, for example. It would be interesting to investigate whether or not evidence of false positive test result rates being higher in the more deprived is still observed when screening with Faecal Immunochemical Test for haemoglobin (FIT) as the initial test, since these tests are not subject to dietary interference. At the time of the analysis presented in this Chapter, of the various studies investigating the impact of deprivation at various stages of the colorectal cancer screening process, none had assessed the relationship with faecal haemoglobin (Hb) concentration when using quantitative FIT.

In the recent assessment of FIT as a first-line test in Scotland, (Steele *et al.*, 2013) it was documented that faecal Hb concentration was higher in men than in women, and increased with age in both genders. (McDonald *et al.*, 2012) It was stated that these data were vital considerations for screening programme design, more tailored strategies were needed and faecal Hb concentration could be included in individual risk-scores along with gender and age. In consequence, since deprivation is of

considerable relevance to colorectal cancer, including test uptake and positivity rates in screening programmes, it is important to also consider the relevance of deprivation as a risk factor. With this in mind, the aim of this Chapter was to investigate the relationship between deprivation and faecal Hb concentration and how this may relate to findings at colonoscopy.

6.2 Materials and methods

The study cohort was again derived from the participants of the 'FIT as a First-Line Test' evaluation, the full process of which was previously described in a previous Chapter (Chapter 3: The relationship between faecal haemoglobin concentration and severity of colorectal neoplasia).

Deprivation was categorized as described in Chapter 4: The relationship between faecal haemoglobin concentration and interval cancers. Distributions of faecal Hb concentration were calculated overall and for men and women in deprivation quintiles. MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software was used for all calculations.

6.3 Results

Of the 66,725 men and women invited, aged 50 to 74 years, faecal Hb concentration was measured on single samples from 38,439 participants who had degree of deprivation calculated, with 48.8% men and 53.3% women. Table 6.1 shows the number in each Scottish Index of Multiple Deprivation (SIMD) quintile, further broken down by gender and age. There were no significant differences in the gender or age distributions in each deprivation quintile ($p > 0.05$).

Table 6.1. Number of participants in each Scottish Index of Multiple Deprivation (SIMD) quintile.

	Deprivation					
	SIMD 1 (most deprived) n (%)	SIMD 2 n (%)	SIMD 3 n (%)	SIMD 4 n (%)	SIMD 5 (least deprived) n (%)	Total n
Total	5,450 (14.2)	7,604 (19.8)	6,780 (17.6)	9,962 (25.9)	8,643 (22.5)	38,439
Gender:						
Men	2,556 (14.3)	3,572 (19.9)	3,171 (17.7)	4,667 (26.0)	3,968 (22.1)	17,934
Women	2,894 (14.1)	4,032 (19.7)	3,609 (17.6)	5,295 (25.8)	4,675 (22.8)	20,505
Age (years):						
50-54	1,473 (16.7)	1,609 (18.2)	1,443 (16.3)	2,497 (28.2)	1,821 (20.6)	8,843
55-59	1,226 (14.1)	1,751 (20.2)	1,602 (18.4)	2,228 (25.7)	1,877 (21.6)	8,684
60-64	1,115 (12.6)	1,806 (20.5)	1,515 (17.2)	2,207 (25.0)	2,176 (24.7)	8,819
65-69	766 (12.6)	1,228 (20.2)	1,149 (18.9)	1,537 (25.3)	1,385 (22.8)	6,065
70-74	870 (14.4)	1,210 (20.1)	1,071 (17.8)	1,493 (24.8)	1,384 (23.0)	6,028

For men and women and for all deprivation quintiles, none of the distributions of faecal Hb concentration were Gaussian (D'Agostino-Pearson test, $p < 0.0001$) and the coefficients of skewness and kurtosis were significantly > 1 ($p < 0.0001$). Of particular note was that the distribution of faecal Hb concentration was highly positively skewed: 51.9% of all participants had no detectable faecal Hb concentration. A significantly greater proportion of those in the least deprived quintile (56.5%) had no detectable faecal Hb concentration than those in the most deprived quintile (45.5%, $p < 0.0001$). Distributions of faecal Hb concentration for each deprivation quintile are displayed as percentiles with conventional 95% confidence interval (CI) in Tables 6.2 and 6.3 for men and women, respectively. The 97.5% percentile represents the potential non-parametric upper reference limit (URL) of the 0.95 inter-fractile reference interval and 90% CI are given as recommended in Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines for derivation of population-based reference values. (Wayne, 2008)

Table 6.2. Percentiles, with 95% confidence intervals (CI), of faecal haemoglobin concentration (µg Hb/g faeces) in men and potential upper reference limits (URL) with 90% CI.

Deprivation	n (%)	25th (95% CI)	50th (95% CI)	75th (95% CI)	90th (95% CI)	95th (95% CI)	97.5th URL (90% CI)
Total	17,934	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.2)	2.3 (2.2 - 2.4)	12.2 (11.4 - 13.4)	37.2 (34.0 - 40.6)	104 (93.8 - 116.2)
SIMD 1 (most deprived)	2,556 (14.3)	0.0 (0.0 - 0.0)	0.4 (0.4 - 0.6)	3.2 (2.8 - 3.6)	18.8 (14.8 - 21.4)	49.6 (39.4 - 67.0)	120.8 (101.4 - 149.8)
SIMD 2	3,572 (19.9)	0.0 (0.0 - 0.0)	0.4 (0.2 - 0.4)	3.0 (2.6 - 3.6)	17.6 (15.0 - 21.4)	51.4 (40.6 - 63.4)	141.6 (107.8 - 199.6)
SIMD 3	3,171 (17.7)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.2)	2.2 (2.0 - 2.6)	12.8 (10.6 - 15.0)	39.2 (30.2 - 47.4)	103.6 (72.2 - 143.6)
SIMD 4	4,667 (26.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.8 (1.6 - 2.0)	9.0 (8.0 - 10.4)	29.2 (23.8 - 36.4)	96.2 (77.0 - 116.2)
SIMD 5 (least deprived)	3,968 (22.1)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.6 (1.4 - 1.6)	8.8 (7.4 - 10.8)	29.2 (24.0 - 34.0)	78.2 (62.0 - 96.4)

Table 6.3. Percentiles, with 95% confidence intervals (CI), of faecal haemoglobin concentration (µg Hb/g faeces) in women and potential upper reference limits (URL) with 90% CI.

Deprivation	n (%)	25th (95% CI)	50th (95% CI)	75th (95% CI)	90th (95% CI)	95th (95% CI)	97.5th URL (90% CI)
Total	20,505	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.6 (1.6 - 1.8)	7.0 (6.6 - 7.4)	21.8 (20.2 - 23.4)	55.6 (50.6 - 62.4)
SIMD 1 (most deprived)	2,894 (14.1)	0.0 (0.0 - 0.0)	0.2 (0.2 - 0.4)	2.4 (2.2 - 2.6)	11.6 (10.0 - 14.8)	33.4 (26.4 - 41.4)	101.2 (71.6 - 126.6)
SIMD 2	4,032 (19.7)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.2)	2.0 (1.8 - 2.0)	8.4 (17.2 - 10.0)	24.8 (20.4 - 29.4)	72.8 (53.2 - 85.6)
SIMD 3	3,609 (17.6)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.6 (1.4 - 1.8)	6.4 (5.6 - 7.4)	19.2 (15.8 - 23.4)	42.4 (32.6 - 58.6)
SIMD 4	5,295 (25.8)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.4 (1.4 - 1.6)	6.0 (5.6 - 6.6)	20.0 (16.0 - 23.4)	52.8 (39.4 - 63.4)
SIMD 5 (least deprived)	4,675 (22.8)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.2 (1.2 - 1.4)	5.6 (5.0 - 6.4)	15.6 (13.0 - 19.2)	40.4 (31.0 - 51.8)

The Krusall-Wallis non-parametric analysis of variance (ANOVA) showed that highly statistically significant variation existed in faecal Hb concentration across deprivation quintiles ($p < 0.000001$), with median faecal Hb concentration increasing as deprivation increased. Post-hoc analysis revealed that those in the least and the second least deprived quintiles had significantly higher median faecal Hb concentration than those in each of the three remaining less deprived quintiles ($p < 0.0001$ for all comparisons).

Table 6.4 shows the proportion of participants with faecal Hb concentration above different cut-off concentrations for a positive test result commonly used in screening programmes, for each deprivation quintile, for men and women. Also estimated were the cut-off faecal Hb concentration that would generate a test positivity rate of 2.0% in each deprivation quintile, as this is the level deemed desirable for referral to colonoscopy for the Screening Programme in Scotland, within the limits of the available colonoscopy resource. The test positivity rate with the cut-off faecal Hb concentration used in the Scottish 'FIT as a First-Line Test' evaluation of 80 µg Hb/g faeces would range from 1.96% in the least deprived up to 3.21% in the most deprived and would be higher in men than women. Logistic regression analysis showed that participants in the most deprived group were more likely to have a faecal Hb concentration above the cut-off faecal Hb concentration for test positivity compared with the least deprived group, independent of gender and age (adjusted odds ratio = 1.70, 95% CI: 1.37 - 2.11). Odds ratios for test positivity across the different degrees of deprivation are displayed in Table 6.5, both non-adjusted and adjusted for gender and age.

Table 6.4. Test positivity rates (%) at commonly used cut-off faecal haemoglobin (Hb) concentrations and cut-off to attain 2.0 % positivity for men and women.

Gender	Deprivation	Faecal Hb concentration (µg Hb/g faeces)					Cut-off for 2% positivity (µg Hb/g faeces)
		10	15	20	40	80	
Men:	**Total**	11.2	9.0	7.6	4.7	3.0	136
	SIMD 1 (most deprived)	13.7	11.2	9.5	5.8	3.6	148
	SIMD 2	13.6	11.1	9.4	5.8	3.5	> 200
	SIMD 3	11.4	9.1	7.5	4.9	2.9	150
	SIMD 4	9.4	7.4	6.4	4.0	2.8	124
	SIMD 5 (least deprived)	9.6	7.7	6.1	3.9	2.3	104
Women:	**Total**	8.1	6.2	4.6	3.3	2.0	80
	SIMD 1 (most deprived)	11.2	8.9	7.6	4.3	2.9	126
	SIMD 2	9.2	6.9	4.9	3.5	2.2	92
	SIMD 3	7.7	6.0	4.4	3.2	1.7	66
	SIMD 4	7.1	5.2	3.9	3.2	1.9	70
	SIMD 5 (least deprived)	6.8	5.2	3.7	2.6	1.6	58

Table 6.5. Odds ratios with 95% confidence interval (CI) for test positivity for each Scottish Index of Multiple Deprivation (SIMD) quintile.

Deprivation	Positivity rate	Odds ratio	
		Non-adjusted (95% CI)	Adjusted (95% CI)*
Total:			
SIMD 1 (most deprived)	3.21%	1.66 (1.34 - 2.06)	1.70 (1.37 - 2.11)
SIMD 2	2.83%	1.46 (1.19 - 1.79)	1.46 (1.19 - 1.79)
SIMD 3	2.23%	1.14 (0.91 - 1.43)	1.14 (0.91 - 1.42)
SIMD 4	2.33%	1.20 (0.98 - 1.46)	1.21 (0.99 - 1.48)
SIMD 5 (least deprived)	1.96%	1.00	1.00
Men:			
SIMD 1 (most deprived)	3.56%	1.54 (1.15 - 2.06)	1.60 (1.19 - 2.15)
SIMD 2	3.50%	1.51 (1.15 - 1.98)	1.53 (1.16 - 2.01)
SIMD 3	2.87%	1.23 (0.92 - 1.65)	1.24 (0.92 - 1.66)
SIMD 4	2.83%	1.21 (0.93 - 1.57)	1.21 (0.95 -1.62)
SIMD 5 (least deprived)	2.34%	1.00	1.00
Women:			
SIMD 1 (most deprived)	2.90%	1.81 (1.32 - 2.48)	1.83 (1.33 - 2.50)
SIMD 2	2.23%	1.38 (1.02 - 1.88)	1.37 (1.01 - 1.86)
SIMD 3	1.66%	1.02 (0.73 - 1.44)	1.02 (0.73 - 1.44)
SIMD 4	1.89%	1.16 (0.86 - 1.57)	1.18 (0.88 - 1.48)
SIMD 5 (least deprived)	1.66%	1.00	1.00

* - adjusted for gender and age quintile, as appropriate.

Detection of advanced neoplasia in participants with a faecal Hb concentration above the cut-off adopted was also examined. Analysis of all screening participants show that the odds ratio for having a positive test result and then having advanced neoplasia detected rose with each increasing deprivation quintile compared with the least deprived, albeit not to statistical significance (adjusted odds ratio in most deprived: 1.48 (95% CI: 0.99 - 2.22). However, no trend at all was apparent for increasing likelihood

of advanced neoplasia detection with participants with a positive test result. This translates as no association existing between deprivation and PPV for advanced neoplasia, and, as such, no association with the number of false positive test results. Table 6.6 shows PPV for colorectal cancer, higher-risk adenoma, and other pathology calculated for each deprivation quintile, by gender. Overall PPV for advanced neoplasia were slightly higher in the least deprived, but the differences did not reach statistical significance ($p > 0.05$).

Table 6.6. Positive Predictive Values (PPV) for each deprivation quintile, by gender.

SIMD	1 (most deprived)		2		3		4		5 (least deprived)	
	n	PPV	n	PPV	n	PPV	n	PPV	n	PPV
All positive test results:										
All	153		183		130		194		154	
Men	80		105		78		112		84	
Women	73		78		52		82		70	
Cancer (CRC):										
All	8	5.2%	4	2.2%	10	7.7%	7	3.6%	10	6.5%
Men	5	6.3%	3	2.9%	6	7.7%	3	2.7%	6	7.1%
Women	3	4.1%	1	1.3%	4	7.7%	4	4.9%	4	5.7%
Higher-risk adenoma (HRA):										
All	36	23.5%	42	23.0%	36	27.7%	39	20.1%	37	24.0%
Men	22	27.5%	28	26.7%	22	28.2%	30	26.8%	25	29.8%
Women	14	19.2%	14	17.9%	14	26.9%	9	11.0%	12	17.1%
Advanced neoplasia (CRC + HRA):										
All	44	28.8%	46	25.1%	46	35.4%	46	23.7%	47	30.5%
Men	27	33.8%	31	29.5%	28	35.9%	33	29.5%	31	36.9%
Women	17	23.3%	15	19.2%	18	34.6%	13	15.9%	16	22.9%
Non-neoplastic pathology*/no pathology detected:										
All	108	70.6%	136	74.3%	83	63.8%	145	74.7%	107	69.5%
Men	52	65.0%	73	69.5%	50	64.1%	78	69.6%	53	63.1%
Women	56	76.7%	63	80.8%	33	63.5%	67	81.7%	54	77.1%

* - Non-neoplastic pathology comprises of hyperplastic polyps and other conditions including diverticular disease, haemorrhoids and inflammatory bowel disease.

6.4 Discussion

Deprivation and faecal Hb concentration was examined in a large group of ostensibly asymptomatic people aged 50 – 74 years. As degree of deprivation increased, median faecal Hb concentration increased. Although men had higher concentrations than women in all deprivation quintiles, the trend for increasing odds ratio for faecal Hb concentration above the cut-off of 80 µg Hb/g faeces with increasing deprivation seemed slightly stronger in women.

The relationship between faecal Hb concentration and deprivation is reflected by those in the more deprived groups having a higher proportion of participants with faecal Hb concentration above the cut-off used in the 'FIT as a First-Line Test' evaluation in Scotland (Steele *et al.*, 2013), in men and in women. The same was seen at all four of the other commonly-used screening cut-off faecal Hb concentration examined, in men and in women, showing that the findings would apply in the same way if lower cut-off faecal Hb concentration were implemented rather than the relatively high 80 µg Hb/g faeces cut-off adopted in the evaluation. This cut-off was deliberately selected with an aim to generate an overall test positivity rate of around 2%, but in reality resulted in the referral of a slightly higher 2.44% of participants to colonoscopy. However, test positivity rates ranged from 1.96% to 3.21% between the lowest and highest quintile of deprivation, respectively; this in real terms would represent a sizeable difference in the number of people referred from the different deprivation quintiles if taking into account the entire screening population.

If equality of test positivity rates were to be achieved across deprivation groups, varying cut-off faecal Hb concentration would have to be employed for each group. This seems particularly evident in women in this analysis, with the cut-off faecal Hb

concentration required to generate 2% test positivity in the most deprived women being more than double the concentration that would give this same referral rate in least deprived women. Crucially though, it would not be the case that adapting the cut-off faecal Hb concentration for these different groups is an appropriate strategy to address any inequalities between in terms of colorectal cancer incidence and mortality. What is of paramount importance is minimising numbers of missed cases of advanced neoplasia in each group while limiting the number of screening participants being referred for unnecessary colonoscopy. Therefore, measures of sensitivity and specificity for men and women in the varying degrees of deprivation would allow a more comprehensive analysis of how any group might be disadvantaged by the use of a single cut-off faecal Hb concentration for all. Since colorectal disease status was known only for those attending follow-up for positive screening test results, and not those with faecal Hb concentration below the cut-off concentration, such analysis was not possible. What could be examined, however, were the PPV for neoplasia in each deprivation quintile.

In contrast to results of previous studies showing lower PPV for neoplasia and, in turn, higher false positive rates in more deprived groups participating in gFOBT screening (Mansouri *et al.*, 2013) no significant differences in these rates were detected between the deprivation quintile in those with a positive test result in this cohort. It could be speculated that the variation in false positive rates by deprivation when screening with gFOBT was in part attributable to a greater consumption of red meat, for example, in the more deprived. Since FIT are not at all subject to dietary interference, this driving mechanism may be have been negated in this cohort. Indeed, our results are now supported by very up-to-date work from Australia, (Symonds *et al.*, 2015b) albeit with a much lower cut-off faecal Hb concentration used than in the Scottish evaluation. Symonds *et al.* (2015b) observed a trend of increasing faecal Hb concentration with

increasing deprivation, and reported that those in the lowest socioeconomic status quintile had a significantly lower odds ratio for test positivity with a cut-off faecal Hb concentration of 20 µg Hb/g faeces than those in the most deprived group (odds ratio = 0.65, 95% CI: 0.53 – 0.80, $p < 0.001$). No significant differences were seen in false positive rates, defined as the absence of any neoplasia, between the deprivation quintiles. The same study also reported that faecal Hb concentration and test positivity were significantly higher in those who had previously participated in colorectal cancer screening ($p < 0.05$), although false positivity rates showed no association with previous screening. Since the number of false positive test results will fall with increasing cut-off faecal Hb concentration, the work presented in this Chapter provides further evidence of this relationship in a country with limited colonoscopy capacity and may be of concern to screening programme organisers.

A plausible explanation is that the more deprived experience more colonic bleeding than the least deprived, whether it is arising from neoplastic lesions or for other less serious reasons. It may be that colorectal cancer incidence is higher in all participants in the most deprived quintiles, and this coupled with bleeding perhaps arising as a result of lifestyle choices associated with deprivation such as a low fibre diet and increased alcohol intake for example, contributes to the higher test positivity rates seen in these groups. A further contributing factor to higher test positivity rates in the more deprived could be pointed to by the trend of decreasing uptake of screening with increasing deprivation (Digby *et al.*, 2013). Delayed participation may therefore be a factor, with more participants in the more deprived groups responding to screening for the first time, or responding less regularly than the less deprived groups. Modification of the cut-off concentration to achieve equality of test positivity rates would be detrimental to improving colorectal cancer detection in this case. Raising the cut-off faecal Hb concentration for the most deprived may miss cases of neoplasia in that

group, with no benefit to the least deprived. Likewise, lowering of the cut-off faecal Hb concentration for the least deprived may result in improved disease detection rates in this group, but would offer no corresponding benefit to the most deprived and therefore possibly only act to widen an already existing disparity in colorectal cancer mortality between deprivation quintiles.

Since sensitivity and specificity measures cannot be calculated here, the results from this cohort cannot form any firm basis for recommendations regarding tailoring of cut-off faecal Hb concentration according to degree of deprivation. However, the trend of increasing odds of having a positive screening test result, then having advanced neoplasia detected, with increasing degree of deprivation (controlling for the confounding effects of age and gender) came very close to reaching statistical significance. This is perhaps enough to merit at least the consideration of the inclusion of degree of deprivation into risk-scoring models, despite the absence of an increase in PPV at the cut-off faecal Hb concentration adopted. It is important that such modelling be kept as simple as possible so as to be feasible and acceptable to those involved in the delivery of the screening programme as an efficient way of prioritising those at greatest risk of disease. More obvious factors for inclusion might include easily obtainable variables such as age and gender. However, if degree of deprivation could be easily incorporated, it may improve the predictive qualities of such a model.

In addition to interpretation of these results in terms of colorectal cancer detection in the screening setting, they may also add some further backing to the evidence base for colorectal cancer prevention. As discussed, changes in the relationship between colorectal cancer incidence and deprivation in men in Scotland have been attributable to those in the least deprived groups improving their lifestyle choices, whereas the

most deprived did not (Oliphant *et al.*, 2011). Campaigns incorporating lifestyle change with the aim of colorectal cancer prevention may wish to target deprived groups in particular with the rationale that they exhibit a greater degree of faecal Hb concentration, which in turn is related to increased severity of colorectal neoplasia. Furthermore, the relationship between faecal Hb concentration and all-cause mortality shown in the important paper by Chen *et al.* (2013) may imply that faecal Hb concentration is representative of overall state of health, with poorer health outcomes in the most deprived reflecting this.

A limitation of this work is that SIMD is an area-based measurement meaning that assumptions may be made of individuals residing in each SIMD "datazone". It is important to mindful of this when interpreting the findings that individual screening participants cannot be labelled as deprived or not based on the area they live in. SIMD more simply reports the relative deprivation of one area compared to another. However, since gathering individual level data on health behaviours, e.g. via questionnaire, would be unfeasible in the setting of population screening, the SIMD index is the best measure available and reasonable conclusions can still be drawn from this analysis.

To summarise, a clear relationship exists between degree of deprivation and faecal Hb concentration. Although likely related to lifestyle factors associated with increasing deprivation, further work is warranted to investigate the mechanisms. With some association existing between deprivation and increased colorectal cancer incidence and poorer outcomes, these data further highlight the need for further work to assist consideration of the adoption of better strategies for setting cut-off faecal Hb concentration, albeit without full knowledge of disease prevalence in participants with

faecal Hb concentration below the cut-off selected for a positive test result. Moreover, these findings show that there is potential for deprivation to be included, along with faecal Hb concentration, gender and age, in the risk-scoring models that are of ever-growing interest. However, it is important to state that although these results in a large cohort document statistically significance variation in faecal Hb concentration according to degree of deprivation, their clinical significance in population screening must be further tested.

7. Faecal haemoglobin concentration as an indicator of significant colorectal disease in patients presenting to primary care with colorectal symptoms

7.1 Introduction

Colorectal symptoms such as diarrhoea, rectal bleeding and abdominal pain are common reasons for patients to present to their general practitioner (GP) in the United Kingdom, with one study reporting that these complaints to account for 10% of all of the clinical work of the NHS (Jones *et al.*, 2009). National Institute for Health and Care Excellence (NICE) (2015) guidelines highlight the symptoms most suggestive of the presence of serious pathology and in need of further investigation by endoscopy. These include rectal bleeding, a mass on examination, iron-deficiency anaemia, but also non-specific symptoms such as a persistent change in bowel habit. However, the same symptoms can often be associated with much less serious causes such as haemorrhoids and irritable bowel syndrome (IBS). The latest revisions of the NICE guidelines (2015) now concede that symptoms have a Positive Predictive Value (PPV) for colorectal cancer of only 3-4%. Indeed, such symptoms alone have been shown in the detailed review and meta-analysis performed by Jellema *et al.* (2010) to show poor diagnostic performance as predictors of colorectal cancer. Only weight loss and iron-deficiency anaemia showed fairly high values for specificity (median 89%, range 72 - 96 and median 92%, range 83 - 95, respectively) but sensitivity was lacking. Therefore, it is difficult for GP to make decisions on which patients are most likely to

have significant colorectal disease encompassing colorectal cancer, higher-risk adenoma and inflammatory bowel disease, and as such should be prioritised for referral for further investigations.

Recent campaigns have run in the United Kingdom with the aim of earlier detection of colorectal cancer. Analysis of the impact of the "Be Clear on Cancer" campaign in England has highlighted the problems associated with placing an emphasis on urgent referral of patients presenting with large bowel symptoms, despite evidence of their poor predictive value. (Peacock *et al.*, 2013) An escalation in the number of referrals for investigation was reported with an increase of 60%, but no increase in colorectal cancer detection was observed, nor was there a stage shift towards earlier diagnosis. In addition, the cost of diagnosing a case of colorectal cancer was calculated to have increased by 30%. A similar campaign exists in Scotland, namely "Detect Cancer Early", with a key aim to encourage those with potential symptoms of colorectal cancer to attend their GP at the earliest opportunity. (Scottish Government, 2014)

In NHS Tayside, the number of referrals for investigation of colorectal symptoms increased from 1,200 per year in 2007 to 4,200 per year in 2013. (Personal communication, Mowat C., 2015) Again, the rapid increase in referrals did not associate with a greater number of colorectal cancer cases diagnosed. Around 35-40% of referrals in NHS Tayside are marked as "Urgent", or "Urgent Suspected Cancer" and, following triage by Consultant Gastroenterologists, around 75% of all referrals are brought straight to investigation with the remainder being assessed in out-patient clinics. Local audit data have revealed a diagnostic yield of colorectal cancer in patients undergoing colonoscopy at just 2%, and 5% for inflammatory bowel disease. Clearly, the trend of increasing referrals over time is placing a burden on endoscopy

services that is not sustainable, particularly in terms of increasing costs without any improvement in disease outcomes. Moreover, waiting times in those who do have significant colorectal disease are being lengthened by the escalating number of negative “urgent” colonoscopies.

Clearly it would be beneficial to identify new means of reducing the number of unnecessary colonoscopies performed in symptomatic patients. Providing GP with a tool to distinguish between those patients in whom a policy of watchful waiting or referral for a specialist opinion may be more appropriate and those who require prioritisation for investigation is urgently required.

The Faecal Immunochemical Test for haemoglobin (FIT) may represent such a tool with a potential role in this context. Detection of haemoglobin (Hb) in faeces is well established in colorectal cancer screening programmes and the merits of FIT over guaiac faecal occult blood tests (gFOBT) and the potential for faecal Hb concentration to act as a predictor of risk have been discussed at length in this work. When using tests for Hb in faeces for colorectal cancer screening in a healthy population, participants with a negative test result are often advised that cancers can be missed, to be aware of symptoms and attend their GP if concerned. However, in patients presenting with symptoms in primary care, it would be unacceptable for patients to be reassured when there is a reasonable possibility of a false negative test result. Therefore, test sensitivity has far higher importance than in the screening setting and as a result, earlier recommendations have indicated that gFOBT has no context in primary care owing to its poor sensitivity. (National Institute for Health and Care Excellence (NICE), 2011; Scottish Intercollegiate Guidelines Network (SIGN), 2011) However, lacking in the literature is evidence of test performance of FIT in primary care

as a rule-out test for significant colorectal disease, evident when the systematic review by Jellema *et al*. (2010) reporting on the value diagnostic tests for colorectal cancer in primary care did not identify any studies involving FIT in this setting. However, since this publication in 2010, some studies have emerged in this field.

Only one of these, from Scotland, (McDonald *et al.*, 2013) assessed test performance for detecting colorectal cancer, higher-risk adenoma and inflammatory bowel disease, and an overall Negative Predictive Value (NPV) of 88.1% was reported with a cut-off concentration of 10 µg Hb/g faeces in 280 patients awaiting colonoscopy. No individuals with colorectal cancer were associated with faecal Hb concentration below this threshold, although a total of only six cases were diagnosed in the cohort. A recent study from Spain compared the diagnostic accuracy of FIT for colorectal cancer detection with the expert guidelines for referral from NICE and the Scottish Intercollegiate Guidelines Network (SIGN). (Cubiella *et al.*, 2014) With a cohort of 787 patients including 97 colorectal cancer cases, the authors showed a NPV of 97.8% for colorectal cancer when using a cut-off concentration of 20 µg Hb/g faeces, with which a test positivity rate of 30.6% was observed. Parente *et al*. (2012) studied 280 patients awaiting colonoscopy for suspicion of colorectal cancer, with an faecal Hb concentration cut-off equivalent to 20 µg Hb/g faeces and reported a NPV for colorectal cancer of 92.0%.

A retrospective population-based study from Sweden described the impact of a *qualitative* FIT used in primary care in 215 patients with colorectal cancer and adenomas exhibiting high-grade dysplasia and concluded that a negative test result delayed further investigation and missed around 15% of colorectal cancer cases. (Hogberg *et al.*, 2013) Another study investigating the use of a *qualitative* FIT in

primary care was performed in England in 126 patients referred via the ‘rapid access’ colorectal service. Although the authors concentrated on FIT as a rule-in test for colorectal cancer, the NPV was 100% for the 17 colorectal cancer cases in the cohort. (Kaul *et al.*, 2013) Kalimutho *et al*. (2011) examined performance of *qualitative* FIT and faecal calprotectin testing in comparison to faecal DNA test performance in 204 consecutive symptomatic patients referred for colonoscopy. NPV was not reported, but sensitivity and specificity for colorectal cancer detection were 51.9% and 97.9%, respectively. Kok *et al.* (2012) also examined *qualitative* FIT in combination with faecal calprotectin testing. With the cut-off faecal Hb concentration as equivalent to 8 µg Hb/g faeces, NPV for significant colorectal disease was 94% (95% CI: 91 - 96) but the study was limited by small numbers with only 19 cases of colorectal cancer, and diverticular disease was included in the definition of significant colorectal disease.

In summary, previous studies reporting results on the use of FIT in symptomatic patients provide some evidence that faecal Hb concentration may perform well as a rule-out test for significant colorectal disease, with high NPV documented, although many of the sample sizes are small. What is missing, however, is an evaluation of the use of faecal Hb concentration at the point of GP referral for endoscopy, rather than in those awaiting colonoscopy. If promising results were available for such an analysis, they could be translated directly into practice to better target colonoscopy towards those at greatest risk of significant colorectal disease. Such evidence could provide GP with the confidence to utilise faecal Hb concentration as a rule-out test for significant colorectal disease, inclusive of higher-risk adenoma and inflammatory bowel disease in addition to colorectal cancer which is mainly reported alone in the literature.

The existing studies discussed here, other than those of McDonald *et al.*, (2013) and Cubiella *et al.*, (2014) have focussed solely on test performance without considering the potential reduction in referral rate. It would be of great value to identify appropriate cut-off faecal Hb concentration for FIT in primary care that would allow a significant reduction in referral rates and subsequently ease the current strain on resources, without missing an unacceptable number of cases of significant colorectal disease. With this in mind, the aim of this study was to assess the diagnostic accuracy of quantitative FIT in patients presenting to primary care with colorectal symptoms and to identify an appropriate cut-off faecal Hb concentration to rule out significant colorectal disease.

7.2 Materials and methods

This prospective study gained the full support of NHS Tayside GP and was conducted following the STAndards for the Reporting of Diagnostic accuracy studies (STARD) guidelines. (2015) All adult patients referred for investigation of colorectal symptoms in NHS Tayside over a six month period from October 2013 to March 2014 were eligible. The NHS Tayside Colorectal Pathway is a unique, single electronic portal of entry for new referrals with daily vetting by consultant gastroenterologists to triage patients either straight to endoscopy or to the appropriate out-patient clinic. At the point of referring patients to the Colorectal Pathway, GP were prompted to request faecal Hb concentration alongside full blood count, urea and electrolytes and C-reactive protein and record the presenting symptoms via the NHS Tayside electronic test requesting software. If patients had more than one presenting symptom, for the purposes of the present analysis they were attributed only one, in order of decreasing clinical

importance as follows: rectal bleeding, anaemia, diarrhoea, altered bowel habit, abdominal pain, weight loss. The total number of referrals and their urgency were recorded on the referral management software. Practice nurses distributed an OC-Sensor FIT specimen collection device (Eiken Chemical Company, Tokyo, Japan), and a pictorial patient instruction sheet to each participant. Patients were instructed to collect samples from a single faeces and to return the sample immediately to the GP surgery. The samples were returned at room temperature via the GP surgery routine sample collection service (a daily van courier service) to Blood Sciences, Ninewells Hospital and Medical School, and stored at 4^{o}C prior to analysis to ensure stability.

Faecal Hb concentration measurement was performed using a single OC-Sensor io analyser, (Eiken Chemical Co., Ltd, Tokyo, Japan). Inter-run imprecision was assessed with quality control materials (Eiken) in each run: coefficients of variation were 4.6% at 25 µg Hb/g faeces and 3.9% at 93 µg Hb/g faeces over the period of this study. Any faecal Hb concentration sample which was reported by the analytical system as a positive numerical result greater than zero µg Hb/g faeces was considered as a "detectable faecal Hb concentration". Samples with results above the upper analytical limit were not diluted and re-assayed but reported as greater than that upper concentration limit of 200 µg Hb/g faeces. Faecal Hb concentration results were converted from the instrument generated ng Hb/ml buffer to the internationally recommended unit of µg Hb/g faeces by multiplication by 0.2. (Fraser *et al.*, 2012) The laboratory had a total quality management system in place and was accredited to International Organization for Standardization (ISO) 15189 based standards.

Patients referred to endoscopy were investigated within six weeks of referral. The NHS Tayside endoscopy units participate in the accreditation scheme of the Joint

Accreditation Group on GI Endoscopy. Participating clinicians and endoscopists were blind to the faecal test results. All findings were recorded on the endoscopy reporting system by the endoscopist. The diagnoses of colorectal cancer, higher-risk adenoma and inflammatory bowel disease were confirmed following assessment by a gastrointestinal pathologist. Clinical outcomes were collected for all patients who completed the tests and the diagnostic accuracies of faecal Hb concentration for identification of significant colorectal disease were examined. MedCalc statistical software (MedCalc Software, Mariakerke, Belgium) was used for all calculations and to produce distribution plots for faecal Hb concentration. The study was approved by the East of Scotland research ethics committee.

7.3 Results

Over the six-month study period, 2,189 patients were referred for investigation from primary care to the NHS Tayside Colorectal Service, with 1,032 marked as either "urgent" or "urgent suspected cancer". Of those referred, 1,043 patients returned a FIT specimen collection devices. Table 7.1 summarises the details of the final study cohort of 1,023 with a faecal Hb concentration measurement. 55.6% of this group were women and median age was 64 years (range 16-95, [interquartile range] IQR 51 - 74).

Table 7.2 shows median faecal Hb concentration according to gender and age, and proportions of patients above increasing cut-off faecal Hb concentration. Median faecal Hb concentration was significantly higher in those aged 70 years old and over compared with those under 40 years old ($p < 0.02$). More women than men and more younger patients than older patients had undetectable faecal Hb concentration in their

sample. Using a cut-off faecal Hb concentration-off of 10 µg Hb/g faeces, the test result was positive in 22.3% of samples.

Table 7.1. Summary of study cohort with number of participants returning a sample for faecal haemoglobin concentration measurement.

	Total		Men		Women	
	n	%	n	%	n	%
Total referrals to NHS Tayside Colorectal Service during the study period	2,189					
Urgent referrals*	1,032	47.1	**	**	**	**
Returned samples	1,043	47.6	465	**	578	**
Excluded:						
Specimen not suitable for analysis	15	1.4	6	1.3	9	1.6
Specimen returned out with study period	4	0.4	2	0.4	2	0.3
Previous diagnosis of significant bowel disease	1	0.1	0	0.0	1***	0.2
Included in final study cohort:	1,023		457	44.7	566	55.3

* Referrals marked “urgent” or “urgent suspected cancer”.
** Data not available for number of urgent referrals by gender.
*** Previous diagnosis of ulcerative colitis.

The prevalences of referred symptoms in the cohort were altered bowel habit (42.7%), rectal bleeding (33.9%), diarrhoea (16.8%), abdominal pain (11.0%), iron-deficiency anaemia (8.7%), weight loss (0.9%) and a palpable mass (0.3%).

A total of 750 patients (54.7% female, median age 64 years, range: 16 - 90, IQR: 52 - 73) had faecal Hb concentration available and completed bowel investigations and were therefore included in the analysis of test performance. The most common findings at colonoscopy were: normal in 240 (32.0%), diverticular disease in 188 (25.1%), haemorrhoids in 97 (12.9%), low-risk adenoma in 65 (8.7%), higher-risk adenoma in 40 (5.3%), inflammatory bowel disease in 34 (4.5%) and colorectal cancer in 28 patients (3.7%).

Table 7.3 shows the number of patients with each outcome according to the referral symptom, with PPV also shown for each symptom.

Figure 7.1 shows the distribution of the 1,023 faecal Hb concentrations, by clinical outcome, along with those who did not complete bowel investigations. Three patients with colorectal cancer had a faecal Hb concentration below 10 µg Hb/g faeces. Median faecal Hb concentration was significantly higher in those with colorectal cancer compared to those with all other outcomes combined (130.1 µg Hb/g faeces, IQR 35.1 - 200.0 v. 0.4 µg Hb/g faeces, IQR 0.0 - 5.8, $p < 0.0001$), higher-risk adenoma compared with all other non-neoplastic outcomes combined, (6.4 µg Hb/g faeces, IQR 1.3 - 190.8 v. 0.4 µg Hb/g faeces, IQR 0.0 - 4.2, $p < 0.0001$), and inflammatory bowel disease compared with all non-neoplastic outcomes (84.0 µg Hb/g faeces, IQR 3.8 - 200.0 v. 0.3 µg Hb/g faeces, IQR 0.0 - 3.8, $p < 0.0001$).

Since three cases of colorectal cancer were associated with low faecal Hb concentration (< 10 µg Hb/g faeces), and it would be generally considered unacceptable for colorectal cancer to be missed in symptomatic patients presenting in

primary care, performance characteristics of faecal Hb concentration for detection of significant colorectal disease was assessed using a cut-off faecal Hb concentration of any detectable faecal Hb concentration, in addition to performance at 10 µg Hb/g faeces. Table 7.4 displays test performance at both of these cut-off faecal Hb concentration.

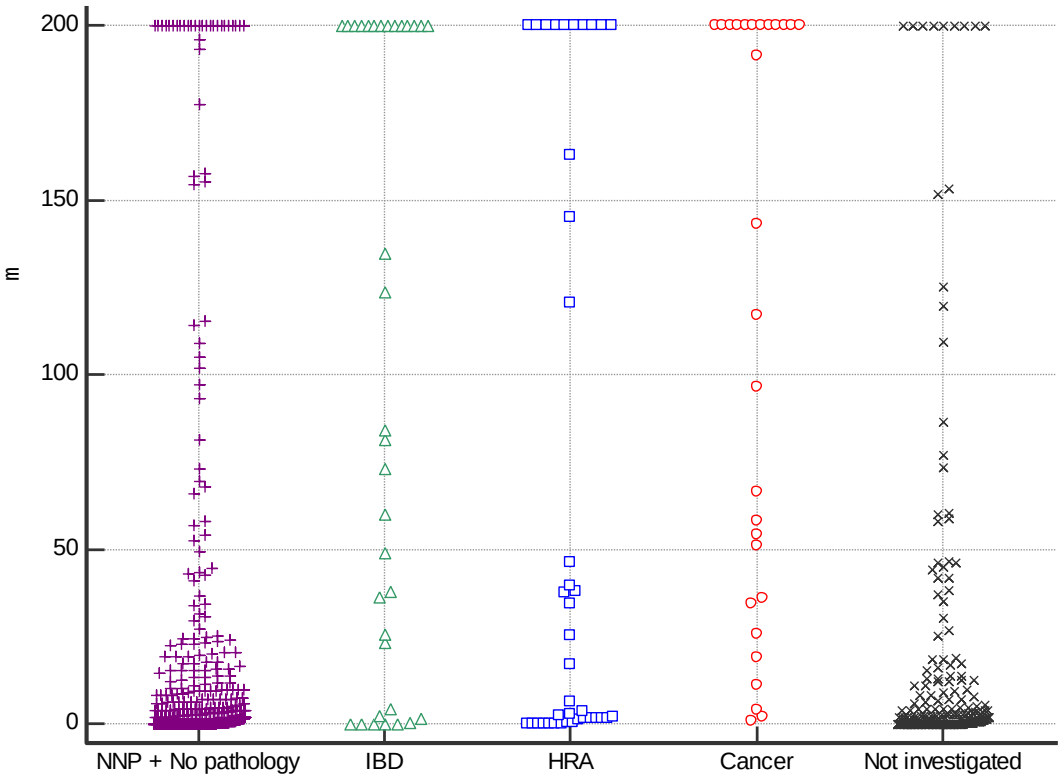
Table 7.2. Number of patients with undetectable faecal haemoglobin concentration (f-Hb), and f-Hb above increasing cut-offs according to gender and age.

	Total	Median f-Hb (µg Hb/g faeces), (IQR)	0 µg Hb/g faeces		> 0 µg Hb/g faeces		≥10 µg Hb/g faeces		≥15 µg Hb/g faeces		≥20 µg Hb/g faeces		≥30 µg Hb/g faeces		≥200 µg Hb/g faeces	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total	1,023	0.4 (0.0-6.6)	434	42.4	589	57.6	228	22.3	198	19.4	174	17.0	153	15.0	70	6.8
Men	457	0.6 (0.0-7.6)	183	40.0	274	60.0	109	23.9	93	20.4	84	18.4	75	16.4	42	9.2
Women	566	0.4 (0.0-6.0)	251	44.3	315	55.7	119	21.0	105	18.6	90	15.9	78	13.8	28	4.9
Age category (years):																
< 40	85	0.2 (0.0-2.7)	40	47.1	45	52.9	17	20.0	15	17.6	13	15.3	12	14.1	7	8.2
40-49	138	0.4 (0.0-2.8)	63	45.7	75	54.3	22	15.9	19	13.8	15	10.9	13	9.4	5	3.6
50-59	190	0.3 (0.0-7.2)	89	46.8	101	53.2	42	22.1	38	20.0	34	17.9	33	17.4	18	9.5
60-69	253	0.4 (0.0-5.9)	116	45.8	137	54.2	54	21.3	42	16.6	36	14.2	29	11.5	15	5.9
≥ 70	357	1.2 (0.0-11.6)	126	35.3	231	64.7	93	26.1	84	23.5	76	21.3	66	18.5	25	7.0

Table 7.3. Symptom prevalence and Positive Predictive Values (PPV) for colorectal cancer (CRC), higher-risk adenoma (HRA), inflammatory bowel disease (IBD) and significant colorectal disease (SCD).

Symptoms	Total		CRC			HRA			IBD			SCD = CRC+HRA+IBD		
	n	%	n	%	PPV	n	%	PPV	n	%	PPV	n	%	PPV
Altered bowel habit	322	42.9	7	25.0	2.2%	13	32.5	4.0%	7	20.6	2.2%	27	26.7	8.4%
Rectal bleeding	256	34.1	11	39.3	4.3%	20	50.0	7.8%	23	67.6	9.0%	54	53.5	21.1%
Diarrhoea	126	16.8	3	10.7	2.4%	6	15.0	4.8%	9	26.5	7.1%	18	17.8	14.3%
Iron-deficiency anaemia	66	8.8	6	21.4	9.1%	1	2.5	1.5%	2	5.9	3.0%	9	8.9	13.6%
Abdominal pain	83	11.1	3	10.7	3.6%	5	12.5	6.0%	2	5.9	2.4%	10	9.9	12.0%
Weight loss	7	0.9	1	3.6	14.3%	0	0.0	0.0%	0	0.0	0.0%	1	1.0	14.3%
Mass	2	0.3	1	3.6	50.0%	0	0.0	0.0%	0	0.0	0.0%	1	1.0	50.0%
Total	750		28			40			34			101*		

Figure 7.1. Distribution of faecal haemoglobin concentration by clinical outcome.



NNP = Non-neoplastic pathology; IBD = inflammatory bowel disease; HRA = higher-risk adenoma.

Table 7.4. Performance of faecal haemoglobin concentration (f-Hb) in the detection of colorectal cancer (CRC), higher-risk adenoma (HRA), all neoplasia (AN), inflammatory bowel disease (IBD) and significant colorectal disease (SCD) using two different cut-off concentrations.

	CRC	HRA	AN = CRC+HRA	IBD	SCD = CRC+HRA+IBD
f-Hb cut-off ≥10 µg Hb/g faeces:					
Positivity rate: 23.5%					
Number	28	40	68	34	102
True positive results	25	20	45	25	70
False negative results	3	20	23	9	32
False positive results	151	156	131	151	106
True negative results	571	554	551	565	542
Positive Predictive Value	14.2%	11.4%	25.6%	14.2%	39.8%
Negative Predictive Value	99.5%	96.5%	96.0%	98.4%	94.4%
Sensitivity	89.3%	50.0%	66.2%	73.5%	68.6%
Specificity	79.1%	78.0%	80.8%	78.9%	83.6%
f-Hb cut-off any detectable blood:					
Positivity rate: 58.3%					
Number	28	40	68	34	102
True positive results	28	33	61	29	90
False negative results	0	7	7	5	12
False positive results	409	404	376	408	347
True negative results	313	306	306	308	301
Positive Predictive Value	6.4%	7.6%	14.0%	6.7%	20.6%
Negative Predictive Value	100%	97.8%	97.8%	98.4%	96.2%
Sensitivity	100%	82.5%	89.7%	85.3%	88.2%
Specificity	43.4%	43.1%	44.9%	43.0%	46.4%

Whilst only small numbers were available, some analysis of test performance by gender was possible. At a cut-off faecal Hb concentration of any detectable Hb, test positivity rate would be higher in men than in women (60.9% v. 56.0%). Using this criteria for referral would give a PPV for colorectal cancer of 6.3% in men and 6.6% in women. However, overall PPV for colorectal cancer, higher-risk adenoma and inflammatory bowel disease would be higher in men, at 22.6%, compared with 18.3% in women. This was due to higher proportions of both higher-risk adenoma and inflammatory bowel disease in men with detectable Hb in their sample compared to women. Interestingly, all three of the colorectal cancer cases with faecal Hb concentration below the other cut-off faecal Hb concentration examined of ≥ 10 μg Hb/g faeces occurred in women. No colorectal cancer would be therefore have been missed in this cohort using this higher cut-off faecal Hb concentration in men, although 12 patients with higher-risk adenoma and five with inflammatory bowel disease would not have been urgently referred compared to seven and fives cases of higher-risk adenoma and inflammatory bowel disease, respectively, in men using the cut-off faecal Hb concentration of any detectable Hb.

Interestingly, 256 of the 755 patients described rectal bleeding, but 87 (34.0%) of these had undetectable faecal Hb concentration. In this subgroup, only 3.4% had significant colorectal disease (two with higher-risk adenoma, one with inflammatory bowel disease), and the most common finding was haemorrhoids, the most severe diagnosis in 32 patients reporting rectal bleeding but having undetectable faecal Hb concentration (36.8%).

Of the 28 patients diagnosed with colorectal cancer, eight were situated in the proximal colon (28.6%), six in the distal colon (21.4%) and 14 in the rectum (50.0%). Although

the numbers are too small to draw any conclusions, no significant differences in median faecal Hb concentration were observed between patients with colorectal cancer located in different regions of the colorectum. All three of the cases of colorectal cancer with a faecal Hb concentration < 10 µg Hb/g faeces were Dukes' stage C. Two were located in the sigmoid colon, with maximum tumour diameters of 20 mm and 50 mm, with the other a 38 mm rectal tumour.

No significant difference was detected in median faecal Hb concentration between those with early stage colorectal cancer compared with late stage colorectal cancer, nor was there a correlation found between faecal Hb concentration and tumour size.

7.4 Discussion

The results of this study demonstrate that faecal Hb concentration can be used in a symptomatic population presenting to primary care with colorectal symptoms to help identify those in need of further investigation. Using detectable faecal Hb concentration as the criterion for referral identified 58.3% of patients as having a positive test result and a PPV for significant colorectal disease of 20.6%. The significant reduction in referral rate is promising in light of the current strain on endoscopy resource in Scotland, but vitally important for GP making decisions on which patients to refer for colonoscopy is that no cases of colorectal cancer are missed. The data from this cohort indicate that GP can use faecal Hb concentration as a decision aid with the confidence that colorectal cancer is effectively ruled out in patients with no detectable faecal Hb concentration, with no cases identified in this cohort using this criterion.

Some cases of higher-risk adenoma and inflammatory bowel disease were diagnosed in patients with undetectable faecal Hb concentration, although this was rare.

Test performance was assessed at the cut-off of any detectable faecal Hb concentration due to three colorectal cancer cases, all late stage, in the cohort having faecal Hb concentration below the more commonly adopted cut-off faecal Hb concentration of 10 µg Hb/g faeces, and missing any colorectal cancer cases in patients reporting colorectal symptoms in primary care would be unacceptable. In keeping with previous findings from colorectal cancer screening data that median faecal Hb concentration is higher in men than in women, and in older than in younger participants, (Fraser & Auge, 2014; Fraser *et al.*, 2014; McDonald *et al.*, 2012; Symonds *et al.*, 2015b) more women than men had undetectable faecal Hb concentration and likewise for younger compared with older patients. Some studies have shown that women have a higher proportion of interval cancer diagnosed in screened populations. (Brenner *et al.*, 2012; Gill *et al.*, 2012; Steele *et al.*, 2012). Therefore, it is perhaps not surprising that all three of the colorectal cancer cases with very low faecal Hb concentration occurred in female patients. This is significant in that it may suggest that colorectal cancer can be ruled out at different cut-off faecal Hb concentration for men and women. Increasing the cut-off faecal Hb concentration from any detectable faecal Hb concentration to ≥ 10 µg Hb/g faeces would greatly reduce the referral rate in men from 60.9% to 25.6%, while improving specificity for all significant colorectal disease from 44.1% to 81.3%. However, these improvements would be associated with a loss in sensitivity from 88.7% to 69.9% and a drop in NPV from 95.5% to 93.2%. Of 22 higher-risk adenoma cases, the number missed would triple from four (18.1%) to 12 (54.5%), and of 18 cases of inflammatory bowel disease, five would be missed (27.8%) rather than just two (11.1%) when using any detectable Hb as the referral criteria.

When considering the impact of undiagnosed higher-risk adenoma in the symptomatic population, it is important to reiterate that not all adenomas will progress to colorectal cancer. In addition, with exception to large adenomas in the rectum, it is unlikely that higher-risk adenoma are responsible for the symptoms that cause patients to present at primary care. Therefore, it could be said that the discovery of higher-risk adenoma in symptomatic populations are largely incidental. In any case, it should be emphasised when proposing that FIT should be used as a decision tool in primary care that this does not mean patients with faecal Hb concentration below the chosen cut-off faecal Hb concentration would never be offered colonoscopy. Patients could potentially complete a repeat test at a later date, with the reasoning that an higher-risk adenoma progressing toward malignancy may associate with colonic blood loss at this time, or that an intermittently bleeding lesion is more likely to be detected at a second testing opportunity. Moreover, a wait-and-wait policy should be implemented in those with an undetectable faecal Hb concentration, where patients can be referred if symptoms persist in the longer term. This is perhaps more relevant for inflammatory bowel disease, since symptoms in these patients are more likely to be a direct result of the condition. Based on symptoms and other aspects relating to the patient, the GP can refer a patient with an undetectable faecal Hb concentration for further assessment at gastrointestinal (GI) clinics in secondary care, and, further to this, a referral to colonoscopy may still made. Therefore, the use of FIT in primary care should not act as a barrier to GP using their own discretion when referring patients in the case of a negative test result at the applied cut-off faecal Hb concentration.

In keeping with comparison of clinical outcomes in screening participants as documented previously in this work, those with colorectal cancer, higher-risk adenoma,

or inflammatory bowel disease had significantly higher median faecal Hb concentration than those with less severe outcomes. This demonstrates that this variation also occurs in a symptomatic population with a cut-off of undetectable faecal Hb concentration applied.

As expected from results of other published studies and the systematic review conducted by Jellema *et al.,* (2010) symptoms were poor predictors of underlying pathology. For example, iron-deficiency anaemia showed good specificity for colorectal cancer at 91.6%, however sensitivity was very poor at 27.3%. However, some further thought on perhaps using symptoms in conjunction with faecal Hb concentration in primary care is warranted. Although possibly incidental, half of all higher-risk adenoma in this cohort were associated with rectal bleeding. Furthermore, rectal bleeding was the most common referral symptom in those with colorectal cancer (39.3%), and occurred in the majority of those who went on to have a new diagnosis of inflammatory bowel disease (67.6%). With the majority of patients with significant colorectal disease reporting rectal bleeding, its status as a “red flag” symptom is seemingly supported. It could be reasonably expected that those reporting rectal bleeding would be least likely to have undetectable faecal Hb concentration in their faeces. However, over a third of these patients had undetectable faecal Hb concentration and a very low proportion of significant colorectal disease was present in this subgroup. This may suggest that reporting of this symptom in primary care is non-specific and the use of faecal Hb concentration in patients with rectal bleeding may still be warranted.

Rectal cancers accounted for half of all colorectal cancer cases. This is a higher proportion than that observed in the screening population discussed in an earlier

Chapter of this work at 35.9%. This might suggest that rectal cancers are more likely to trigger symptoms prompting a patient to attend their GP surgery. An intriguing observation when analysing site distribution of both interval cancer and screen-detected colorectal cancer was that a higher proportion of rectal colorectal cancer occurred in women than men, with 53.3% of all colorectal cancer in women arising at this site compared with 29.0% in men. In the symptomatic population examined here, 11 of the 14 rectal cancers occurred in female patients (78.6%), with 73.3% of all colorectal cancer in women occurring in the rectum compared with just 23.0% in men. With the caveat that numbers are small, this may further indicate that rectal cancers are more prevalent in women than in men. Drawing conclusions from these results is complicated, and further work is required into which biological mechanisms may be driving this trend.

Less than half of the population referred to NHS Tayside's Colorectal Service over the six-month study period returned a sample. Since it was not mandatory for GP to request the test when referring patients for investigation, some GP will have made a personal choice not to provide patients with the kit. As a result, it is not known how many patients did not receive a kit to complete and how many were provided with the opportunity to provide a sample of faeces but were non-compliant. The reduction in referral rate of over 40% if using a cut-off faecal Hb concentration of any detectable faecal Hb concentration in this study was calculated when only taking into account those patients referred for investigations who had submitted a sample. Achieving a similar reduction in primary care if implemented as mandatory to complete the referral would rely on patient compliance. It is a positive finding that, in keeping with observations from the Scottish Bowel Screening Programme's evaluation of quantitative FIT, (Steele *et al.*, 2013) only a small number of samples were unsuitable for analysis. An opportunity to repeat the test could be provided to these patients.

This study adds to the existing investigations into the role of quantitative FIT in primary care, an area that, in comparison to the available literature on test performance in screening populations, is scarce. At the time of this work, only three other studies in this field were identified (Cubiella *et al.*, 2014; McDonald *et al.*, 2013; Parente *et al.*, 2012), with only the last conducted with a reasonably large cohort. More recently, two further studies have emerged, both from Catalonia, Spain, confirming our findings that FIT has a role as a strong rule-out test in symptomatic patients. Auge *et al.* (2015) performed analysis of faecal samples using the HM-JACKarc quantitative FIT system (Kyowa Medex Co., Ltd, Tokyo, Japan) from 208 symptomatic patients undergoing colonoscopy. NPV for advanced neoplasia were calculated as 95.0% and 89.2% using cut-off faecal Hb concentration of detectable blood and 10 µg Hb/g faeces, respectively. The associated test positivity rates at these respective cut-off faecal Hb concentration, however, were rather different to those presented in the work of this Chapter at 90.4% and 15.8%, owing to the analytical system used in the Spanish study having a lower analytical detection limit. Only two cases of colorectal cancer were diagnosed in the cohort, and the authors have confirmed in personal correspondence that neither were associated with undetectable faecal Hb concentration. The other recent study from Catalonia by Rodriguez-Alonso *et al.* (2015) compared test performance of OC-Sensor FIT at various cut-off faecal Hb concentrations with current guidelines for urgent referral in 1,054 patients referred for colonoscopy. NPV of 94.1% and 93.4% were reported at hypothetical cut-off faecal Hb concentration of detectable faecal Hb concentration and 10 µg Hb/g faeces, respectively, with no patients who had colorectal cancer diagnosed having an undetectable faecal Hb concentration. These new studies enhance the small but growing evidence base supporting the role of faecal Hb concentration in symptomatic patients and confirm the results presented in this Chapter. In addition, the results here provide unique data on the use of FIT at the point

of GP referral, therefore allowing for direct implementation of this strategy as a fully-rolled out decision tool, backed up by the knowledge of its feasibility in this setting.

Further, analysis may be of interest concerning the development of algorithms to improve specificity of the referral process using faecal Hb concentration, for example taking into account the age of the patient, bearing in mind that colorectal cancer is very rare in those aged below 50 years, with the youngest patient in the cohort with colorectal cancer being 56 years old. More detailed work is also still required into the use of faecal Hb concentration in combination with symptoms, as well as other risk factors for significant colorectal disease. Although undetectable faecal Hb concentration seems to perform well as a rule-out biomarker for colorectal cancer, and does not miss many higher-risk adenoma and inflammatory bowel disease cases, specificity is poor at 46.4% and only 20.6% of those with detectable faecal Hb concentration had significant colorectal disease. Therefore, a large number of investigations would still be performed in those without demonstrable significant colorectal disease using this criteria. With risk scoring systems now increasingly emerging for use in screening populations, similar models can be developed for implementation in primary care for triage of symptomatic patients for urgent investigation. Such models would need to avoid over complication of the referral process to promote agreeability to their use on the part of GP, with easily-obtainable variables. This is the next step for research into the role of faecal Hb concentration in primary care to reduce the burden on colonoscopy resource caused by unnecessary invasive investigations.

Finally, these findings may have important ramifications in context of the debate surrounding the very recent update to the NICE guidelines for recognition and referral

of suspected colorectal cancer. (National Institute for Health and Care Excellence (NICE), 2015) Previously, the NICE guidelines made no mention of tests for the presence of Hb in the faeces (National Institute for Health and Care Excellence (NICE), 2011) and the advice from SIGN on diagnosis of colorectal cancer was that gFOBT are not indicated for use in the primary care setting and should not impact on the need to investigate symptoms. (Scottish Intercollegiate Guidelines Network (SIGN), 2011) New Healthcare Improvement Scotland (HIS) referral guidelines for suspected cancer, however, state that "a recent negative faecal occult blood test should not rule out the need to refer". (Healthcare Improvement Scotland (HIS), 2015) Contrary to the body's previous advice, the new NICE guidelines (2015) now recommend that faecal tests are used, in the absence of overt rectal bleeding, in those aged 50 years and over with unexplained abdominal pain or weight loss, those aged below 60 years with altered bowel habit or iron-deficiency anaemia, or those aged 60 years and over who have anaemia even in the absence of iron-deficiency anaemia. Urgent referral for suspected cancer, according to the guidelines, should be made in patients whose "tests show occult blood in their faeces".

Several serious reservations were raised by a large group of multidisciplinary figures in response to the updated NICE guidance, (Steele *et al.*, 2015) with one major concern being that it is not stated which faecal test should be utilised. Further concerns were raised by Benton *et al.* (2015) around the number of false negative test results that would occur using gFOBT in primary care and the assumption that such patients would re-present within a year was rejected. An objection was made that the guidelines were based around the PPV of gFOBT for colorectal cancer with not enough emphasis given to the NPV, which has been shown to be as low as 16% with gFOBT in primary care.

The various benefits offered by FIT over gFOBT have been outlined in detail throughout this thesis, with a standout advantage in the context of symptomatic patients being the improved sensitivity that quantitative FIT can offer by way of the option to lower the faecal Hb concentration cut-off to such that can effectively rule-out colorectal cancer whilst significantly reducing the burden on endoscopic resource. For this to be achieved in the cohort described in this Chapter, a far lower cut-off faecal Hb concentration was required than that commonly used in colorectal cancer screening programmes, to eliminate patients with false negative test results who it would be unacceptable to falsely reassure in primary care. Worryingly, this is not possible using gFOBT, with which interval cancer proportions in colorectal cancer screening programmes have been repeatedly demonstrated at upwards of 50%. (Digby *et al.*, 2015; Steele *et al.*, 2012; Tazi *et al.*, 1999; Hardcastle *et al.*, 1996; Kronborg *et al.*, 1996; Faivre *et al.*, 1991) Moreover, symptomatic patients by nature represent a higher risk group than asymptomatic screening populations.

In response to the negative correspondence received, the authors of the updated NICE guidelines stated that it was not specified which test is to be used since FIT did not, at the time of the development of the advice, have any supportive studies in symptomatic patients in primary care. (Hamilton *et al.*, 2015) The evidence supporting the use of FIT as a rule-out test for colorectal cancer at a cut-off faecal Hb concentration of any detectable faecal Hb concentration presented in this Chapter surely supports the argument that quantitative FIT, owing to the feature of an adjustable cut-off faecal Hb concentration, must be the test of choice if faecal tests are to be used to aid the decision of whether or not to refer patients with colorectal symptoms presenting at primary care. Indeed, a very recent editorial by Fraser & Strachan, (2015) also expressing reservations towards the updated NICE guidelines, explained that the growing evidence showing FIT to have applicability to assessment of symptomatic

patients presenting to primary care, including a publication resulting from the work presented in this Chapter, has emerged mainly during and after the publication of the new NICE guidelines. The debate around the current referral guidance will persist for the time being, and more studies demonstrating the value of using quantitative FIT in symptomatic patients in primary care are required to build the evidence base desired by the authors of the guidelines for a revision to be considered.

8. Summary and Conclusions

8.1 Summary of key findings

The findings of this work support the use of faecal haemoglobin (Hb) concentration as an important predictor of significant colorectal neoplasia. Detailed analysis of data arising from the Scottish Bowel Screening Programme's 'FIT as a First-Line Test' evaluation gives backing to the thesis is that faecal Hb concentration can be better optimised in colorectal cancer screening programmes than simply implementing as a binary test with one cut-off faecal Hb concentration used for all. To summarise, median faecal Hb concentration was higher in those with advanced neoplasia than those with less severe outcomes in those with a positive screening test result and that in those with a negative screening test result, an elevated faecal Hb concentration associated with a greater likelihood of a future diagnosis of AN, either as an interval cancer or at the subsequent screening round. A novel finding that faecal Hb concentration shows an independent relationship with degree of deprivation was also demonstrated in the screening population examined. . It was also considered that the use of faecal Hb concentration in primary care would be supported by evidence of high Negative Predictive Values (NPVs) for significant colorectal disease. This was indeed the case, with potential to cut the number of endoscopy referrals by up to 40% based on the absence of detectable faecal Hb, whilst ruling out colorectal cancer in the symptomatic patients studied

8.2 Relationship between faecal haemoglobin concentration and severity of colorectal neoplasia

The results presented in this work further support the existing literature documenting a continuum of risk of colorectal disease with increasing faecal Hb concentration. Using a strong positive guaiac faecal occult blood test (gFOBT) result as an indicator of an elevated concentration of haemoglobin (Hb) in the faeces, evidence of more severe clinical outcomes were observed in comparison to those with less windows on the test card producing a reaction that was positive for the haem component of Hb. Bearing in mind an important area of debate in colorectal cancer screening is whether or not women may be disadvantaged by the use of faecal tests, the gender differences uncovered in the analysis may be significant, particularly with regard to women in both routes to test positivity having later stage colorectal cancer than men. If this points towards earlier stage colorectal cancer being more likely to be missed in women, this may tie in with previous work showing women to have higher interval cancer proportions than men.

More relevant, given the direction that screening programmes are now moving worldwide to use of quantitative Faecal Immunochemical Tests for Hb (FIT), is the exploration of faecal Hb concentration in relation to clinical outcomes. The overall findings in the current work is that faecal Hb concentration relates to lesion size, which in turn relates to risk of malignancy. Although overlap was evident, there was some distinction between the distribution patterns of faecal Hb concentration in the group with advanced neoplasia and those with low-risk adenoma. Although a relatively high cut-off faecal Hb concentration was adopted in this cohort, this finding is promising in terms of the inclination to avoid overdiagnosis of those with small polyps unlikely to

progress to colorectal cancer. It can be speculated that many more such lesions were present in those below the cut-off faecal Hb concentration used. Future work may be warranted on gender and age differences in the relationship between faecal Hb concentration and severity of colorectal neoplasia; this was not possible with the sample size available for analysis.

Even using a relatively high cut-off faecal Hb concentration compared with other studies investigating the relationship between faecal Hb concentration and clinical outcomes, the value of faecal Hb concentration as a predictor of colorectal neoplasia has been further demonstrated. This has implications for programme organisers in countries with a limited colonoscopy resource who may wish to look at targeting colonoscopy towards those considered at greatest risk according to their faceal Hb concentration.

8.3 Faecal haemoglobin concentration as a predictor of interval cancer and advanced neoplasia at the subsequent screening round

With FIT now widely accepted as a more analytically and clinically sensitive test than gFOBT, it would be hoped that FIT could go some way to reducing the high interval cancer proportions commonly reported with gFOBT at around 50%. It seems from the data presented in this work, however, that the use of high cut-off faecal Hb concentration may negate any potential improvements in sensitivity, since an interval cancer proportion of 50.8% was found. The later stage distribution of interval cancer compared with screen-detected colorectal cancer does highlight the benefit of lowering the cut-off faecal Hb concentration, but the fact that almost a fifth of those with an interval cancer diagnosed had undetectable faecal Hb concentration illustrates that

even drastic reductions in the cut-off faecal Hb concentration will not solve the problem of interval cancer within screening programmes using faecal tests for blood.

Further analysis of participants with a negative screening test result was performed to investigate the relationship between faecal Hb concentration and clinical outcomes in the subsequent screening round. The results supported the hypothesis that those with a faecal Hb concentration closer to the cut-off applied were more likely to have advanced neoplasia diagnosed at the subsequent screening round than those with a very low faecal Hb concentration at baseline. This provides further evidence of the predictive power of faecal Hb concentration and perhaps advocates closer future surveillance of participants with a faecal Hb concentration closer to the cut-off. The finding that more false positive test results occurred in those with a previously low f-Hb indicates that colorectal cancer screening programmes may improve their efficiency by prioritising investigation in participants who have elevated f-Hb over consecutive rounds. A clear weakness of this work was that FIT was not the screening test used in the subsequent round in question, since the Scottish Bowel Screening Programme had reverted to the gFOBT/FIT two-tier reflex algorithm following the FIT evaluation. However, with the introduction of FIT now approved in Scotland and due for full roll-out in the near future, an exciting opportunity will exist for investigation of intra-participant variation in f-Hb, and how variation in f-Hb relates to disease status.

8.4 The relationship between faecal haemoglobin concentration and degree of deprivation

With existing evidence linking lower socioeconomic status with increased colorectal cancer mortality, it was of interest to investigate where faecal Hb concentration fits into the relationship. An independent trend of increasing faecal Hb concentration with

increasing degree of deprivation was uncovered. Participants in the most deprived group were more likely to have faecal Hb concentration above the cut-off chosen for the 'FIT as a First-Line Test' evaluation of 80 µg Hb/g faeces, but the higher odds ratio for advanced neoplasia in those in the more deprived groups did not reach statistical significance. Although some potential exists for the inclusion of deprivation as a risk factor in risk scoring models in colorectal cancer screening, its clinical significance is not clear from these findings. It is vital that any risk scoring models are as simple as possible, and the use of deprivation may be an unnecessary complication in this context. A more important focus with regard to deprivation may be better targeting of initiatives to improve uptake in those in more deprived areas, with participation consistently lowest in this demographic. Since faecal Hb concentration is related to all-cause mortality, it might be that the association of faecal Hb concentration and deprivation might reflect overall health; the interesting possibility that faecal Hb concentration may be a modifiable biomarker of this would be very interesting to investigate.

8.5 The role of faecal haemoglobin concentration in primary care

The use of FIT in primary care to distinguish between those with significant colorectal disease and those with less severe, or no pathology is still controversial. Differences in the acceptability of the test exist in this setting of individual testing as opposed to in population screening. The harms associated with a false positive test result such as complications arising at colonoscopy will be more tolerable in a symptomatic population reporting to GP with a view to such investigations than those who perceive themselves to be healthy up until notification of a positive test result. Therefore, a very low cut-off generating a high positivity rate can be introduced in the symptomatic population, that would be considered to upset the balance of harms and benfits of screening. In

addition, false negative test results if found to exhibit a very low chance of false negative test result in a patient with colorectal cancer. However, due to the unsustainable burden being placed on investigative services in the UK as a result of the escalating numbers of urgent referrals from primary care, with a low yield of significant bowel disease, the findings presented in this work support the argument that FIT has a very significant role to play. In simple terms, it was demonstrated that using a cut-off of any detectable faecal Hb concentration would have reduced the referral rate by over 40%, whilst providing general practitioners (GP) with confidence that no cases of colorectal cancer would have been missed. FIT also performed well as a rule-out test for higher-risk adenoma and inflammatory bowel disease, despite some cases having an undetectable faecal Hb concentration. In real terms, such patients may still undergo endoscopy if symptoms persist, and in some cases the FIT test may be bypassed where patients have particularly worrying symptoms to expedite the referral process.

These are very promising results, and it is hoped that they can guide decision makers to incorporate FIT into the referral process for patients presenting at primary care with colorectal symptoms. However, opportunity for further work in this area exists. Although the use of FIT in primary care would make a significant contribution towards alleviating some of the burden on investigative services, a large number of unnecessary colonoscopies would still be performed as demonstrated by the large number of patients who had a detectable faecal Hb concentration, but had no diagnosis of clinically important disease; the PPV for significant colorectal disease using a cut-off of any detectable faecal Hb concentration was just 20.6%. The next step is development of a model to allow more sophisticated stratification of risk than that afforded with faecal Hb concentration alone. Easily obtainable risk factors such as age, gender, specific symptoms and indices from full blood count could be combined

with data on lifestyle and Body Mass Index (BMI), for example, to allow development of a practical scoring tool to identify patients most likely to harbour significant colorectal disease. This may help to distinguish between the very small number of patients with a low or undetectable faecal Hb concentration who had significant colorectal disease and those in whom invasive investigations are unnecessary. Further work to clarify several important issues regarding potential use of FIT in assessment of the symptomatic was called for in a very recently published editorial article (Fraser & Strachan, 2015) in light of the updated National Institute for Health and Care Excellence (NICE) guidelines for recognition and referral of suspected colorectal cancer. (National Institute for Health and Care Excellence (NICE), 2015) The issues raised included how clinical outcomes might differ using different quantitative FIT analytical systems, whether or not the focus should be on detection (rule-in) or eliminating (rule-out) colorectal disease, the role of faecal Hb concentration in clinical pathways to ensure patients with “red-flag” symptoms can still be immediately referred, and the correct management of patients with negative test results but ongoing symptoms. Therefore, there is large scope for future research into the best use of faecal Hb concentration in the primary care setting.

8.6 Overall conclusions

This work supports the hypothesis that faecal Hb concentration can act as a predictor of colorectal neoplasia. The potential for utilisation of faecal Hb concentration measurements below the cut-off used to determine risk of future diagnosis of advanced neoplasia, either as an interval cancer or diagnosed at the subsequent screening round, demonstrates the huge potential that exists with FIT; this is in stark contrast to the far more restrictive nature of gFOBT. Therefore, FIT is the test of choice in screening programmes worldwide as the principle initial screening test before the gold-standard colonoscopy.

It is important to appreciate that the results reported in this thesis - all obtained using the OC-Sensor FIT (Eiken Chemical Co, Japan) - may have been different if using an analytical system from another manufacturer. For example, the lower limit of quantitation varies between manufacturers, meaning that the number of individuals with undetectable faecal Hb concentration reported throughout this work may not be transferable across different systems. Nevertheless, some important messages can be drawn with regard to the relationship between faecal Hb concentration and colorectal neoplasia both in screening and symptomatic populations.

With full implementation of quantitative FIT for screening now approved in Scotland to replace the current gFOBT/FIT two-tier reflex algorithm, many further opportunities will exist for future research with the objective being to uncover strategies that may improve sensitivity for advanced neoplasia without having a negative impact on specificity and the number of false positive test results. The fact that a proportion of interval cancer cases were associated with low faecal Hb concentrations, as were some colorectal cancer cases in the symptomatic population investigated, shows that the test will never operate perfectly and some cases of colorectal cancer will always be missed in screening. However, it is clear that it would be desirable to lower the faecal Hb concentration cut-off to reduce the proportion of interval cancer arising in the screened-population. Currently, the deliberate restriction of the test positivity rate to around 2% both in the Scottish evaluation of FIT screening and through the two-tier algorithm derived with gFOBT and qualitative FIT is driven by constraints on the available colonoscopy resource in Scotland. However, it is hoped that as a result of the promise shown by data from pilot study of FIT utility in primary care, where it seems that urgent referrals could be reduced by up to 40%, some of the colonoscopy resource

could be redirected towards colorectal cancer screening or surveillance of known or previous colorectal disease.

Further scope also exists for investigation into potential strategies to improve efficiency of the colorectal cancer screening programme, through making better use of the adjustable cut-off afforded by quantitative FIT. One interesting area is the concept of reducing the cut-off faecal Hb concentration and extending the screening interval. Some preliminary analysis would be possible with the screen-detected colorectal cancer, interval cancer, and colorectal cancer cases detected at the subsequent screening round to allow a hypothetical calculation of the number of cases that would be detected at an initial screening round with a much lower cut-off faecal Hb concentration than that used in the evaluation, with the subsequent round after two years then missed. However, a longer follow-up period is required than is currently available and a weakness would exist with the return of the Scottish programme to the use of gFOBT. The forthcoming adoption of FIT into the Scottish Programme will allow a better study design to investigate the potential benefits of lowering the cut-off faecal Hb concentration and extending the screening interval.

Further work is also warranted to examine, with greater statistical power, the trends that are emerging in some publications with regard to site differences between interval cancer and screen-detected colorectal cancer. No significant differences were detected in the cohort examined in this work, but the sample size was small. It is interesting that women had a far higher overall proportion of rectal colorectal cancer than men, and it would be intriguing to determine if some biological factor is responsible for this, or if it was more simply an anomaly that was unique to the cohort studied on this occasion. With theories previously offered that colorectal cancer at this

site may associate with faster tumour growth rates, and also that blood arising in the rectum is more likely to contain non-haemolysed erythrocytes, further sub-site analysis is area deserving of future research. Likewise, detailed pathological analysis of factors which may show a more common association with interval cancer compared to screen-detected colorectal cancer can be performed to uncover reasons why some lesions associate with very low faecal Hb concentrations. Anomalies will always occur, but a greater understanding of why some colorectal cancers are missed may drive novel detection strategies. It is possible that further investigation of test sensitivity according to lesion sub-site using a large cohort can be conducted following full roll-out of FIT within the Scottish Bowel Screening Programme.

More comprehensive analysis of any gender inequalities occurring with FIT screening is justified. Much debate exists within the screening community around the use of individualised cut-off faecal Hb concentrations according to gender. A recommendation made in a review from Australia strongly encourages researchers to publish results according to gender where possible; (Massat *et al.*, 2013) this recommendation should be followed to build the evidence base around gender differences in colorectal cancer screening programmes using FIT.

Finally, perhaps the strongest message arising from the work presented in this thesis is that faecal Hb concentration should be incorporated as the major risk factor in risk scoring models developed for use in colorectal cancer screening. With some existing scoring models already showing promising results for prediction of risk when incorporating factors such as age, gender, family history, BMI and lifestyle data, it seems that faecal Hb concentration can greatly add to their power. This is backed up by the sizeable odds ratio calculated in this work for advanced neoplasia in elevated

categories of faecal Hb concentration compared to those with low, or undetectable faecal Hb concentration. On this basis, faecal Hb concentration can no longer be ignored as a strong predictor of risk of advanced neoplasia, and should now be better utilised both in screening and symptomatic populations.

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Appendix: Contributions to the Work in this Thesis

Chapter 2: The relationship between results with the guaiac faecal occult blood test/Faecal Immunochemical Test two-tier reflex screening algorithm and severity of colorectal neoplasia

Results published as: Fraser, C. G., Digby J., McDonald P. J., Strachan J. A., Carey F. A., Steele R. J. C. (2012) Experience with a two-tier reflex gFOBT/FIT strategy in a national bowel screening programme. *J Med Screen*;*19*(1):8–13.

JD obtained the data from the Bowel Screening Scotland (BoSS) IT system, Central Vision laboratory information system and UniSoft endoscopy IT system, performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and participated in the preparation of the manuscript.

CGF (second supervisor) conceived the study and wrote the first draft of the paper, PJMcD supervised the analytical performance of the gFOBT and FIT, JAS was consultant in charge of the Scottish Bowel Screening Centre Laboratory, FAC was consultant pathologist responsible for the pathology and histology and RJCS (first supervisor) was Clinical Director of the Scottish Bowel Screening Programme. All authors saw drafts of the paper and participated in preparation of these and approved the final version.

For the thesis chapter, JD recast the published work with more focus on the comparison of the clinical outcomes of those with an initial weak positive guaiac faecal occult blood test (gFOBT) result with those with a strong positive gFOBT result. The Introduction was rewritten and expanded to explain how this focus could act as a surrogate marked for comparison of those with elevated faecal haemoglobin concentration. The Results and Discussion sections were also expanded to show new analysis and interpretation including gender differences and odds ratios for advanced neoplasia detection and stage and site of colorectal cancer between the routes to positivity.

Chapter 3: The relationship between faecal haemoglobin concentration and severity of colorectal neoplasia

Results published as: Digby, J., Fraser, C. G., Carey, F. A., McDonald, P. J., Strachan, J. A., Diament, R, H., Balsitis, M. & Steele, R. J. C. (2013) Faecal haemoglobin concentration is related to severity of colorectal neoplasia. *J Clin Pathol, 66*(5), 415-419.

JD conceived the study, obtained the data from Central Vision laboratory information system and UniSoft endoscopy IT system, performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and wrote the first draft of the paper with CGF and participated in the preparation of the manuscript.

CGF (second supervisor) played a major role in the generation of the faecal haemoglobin concentration data and wrote the first draft of the paper with JD, FAC was consultant pathologist responsible for the pathology and histology in NHS Tayside, PJMcD was Scottish Bowel Screening laboratory Team Leader and supervised the analytical performance of the gFOBT and Faecal Immunochemical Test for haemoglobin (FIT), JAS was consultant in charge of the Scottish Bowel Screening Centre Laboratory, RHD was Director of NHS Ayrshire & Arran's Bowel Screening Programme, MB was consultant pathologist responsible for the pathology and histology in NHS Ayrshire & Arran and RJCS (first supervisor) was Clinical Director of the Scottish Bowel Screening Programme. All authors saw drafts of the paper and participated in preparation of these and approved the final version.

For the thesis chapter, JD recast the paper to expand and update the review of the existing literature. Results were displayed in greater detail and included multivariate analysis to show odds ratios for colorectal cancer and advanced neoplasia according to faecal haemoglobin categories, gender, and age categories. An expanded Discussion section was also written.

Chapter 4: The relationship between faecal haemoglobin concentration and interval cancers

Results published as: Digby, J., Fraser, C. G., Carey, F. A., Lang, J., Stanners, G. & Steele, R. J. C. (2015) Interval cancers using a quantitative faecal immunochemical test for haemoglobin (FIT) when colonoscopy capacity is limited. *J Med Screen*. pii: 0969141315609634. [Epub ahead of print]

JD obtained the data on the screen-detected cancers UniSoft endoscopy IT system, performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and wrote the first draft of the paper with CGF and participated in the preparation of the manuscript.

CGF (second supervisor) played a major role in the generation of the faecal haemoglobin concentration data and wrote the first draft of the paper with JD, FAC was consultant pathologist responsible for the pathology and histology in NHS Tayside, JL and GS derived the data for the interval cancers and RJCS (first supervisor) was Clinical Director of the Scottish Bowel Screening Programme and initiated the project. All authors saw drafts of the paper and participated in preparation of these and approved the final version.

For the thesis chapter, JD recast the paper to expand all sections. This included the Results being displayed in greater detail including graphics to show distribution of the faecal haemoglobin concentrations associated with all colorectal cancer cases and proportions of interval cancers found within ranges of faecal haemoglobin concentrations. A much expanded Discussion section was also written including in depth interpretation and speculation as to the reasons for gender differences in site and the implications for the Scottish Bowel Screening programme with full roll-out of the Faecal Immunochemical Test for haemoglobin.

Chapter 5: The relationship between faecal haemoglobin concentration and detection of advanced colorectal neoplasia in the subsequent screening round

JD conceived the study, obtained the data from the Bowel Screening Scotland (BoSS) IT system, Clinical Portal information system and Endoscopy Management System (EMS) IT system, performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and wrote the Chapter.

CGF (second supervisor) played a major role in the generation of the faecal haemoglobin concentration data, FAC was consultant pathologist responsible for the pathology and histology in NHS Tayside, RHD was Director of NHS Ayrshire & Arran's Bowel Screening Programme, MB was consultant pathologist responsible for the pathology and histology in NHS Ayrshire & Arran and RJCS (first supervisor) was Clinical Director of the Scottish Bowel Screening Programme.

Chapter 6: The relationship between faecal haemoglobin concentration and degree of deprivation

Results published as: Digby, J., McDonald P.J., Strachan J. A., Libby G., Steele, R. J. C. & Fraser, C. G. (2014) Deprivation and faecal haemoglobin: implications for bowel cancer screening. *J Med Screen*. *21*(2), 95-97.

JD conceived the study, obtained the data from the Bowel Screening Scotland (BoSS) IT system, Clinical Portal information system and Endoscopy Management System (EMS) IT system, retrieved Scottish Index of Multiple Deprivation (SIMD) quintiles,

performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and wrote the paper.

PJMcD supervised the sample handling and analytical performance of the FIT. GL was a Research Statistician at the Scottish Bowel Screening Research UnitGL obtained data, analysed data and contributed to manuscript. JAS was consultant in charge of the Scottish Bowel Screening Centre Laboratory supervised the sample handling and analytical performance of the FIT. RJCS and CGF led the gaining of funding and initiated the project. All authors saw drafts of the paper and participated in preparation of these and approved the final version.

For the thesis chapter, JD wrote a much expanded version than the published short paper version. Results were displayed in greater detail and included multivariate analysis to show odds ratios for a positive screening test result and Positive Predictive Values (PPVs) for advanced neoplasia for each deprivation quintile. An expanded Discussion section was written including the work in context of the aims of the thesis and other recently published work.

Chapter 7: Faecal haemoglobin concentration as an indicator of significant colorectal disease in patients presenting to primary care with colorectal symptoms

Results published as: Mowat, C., Digby, J., Strachan, J. A., Wilson, R., Carey, F. A., Fraser, C. G. & Steele, R. J. C. (2015) Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel

symptoms. *Gut*. Published Online First: doi:10.1136/gutjnl-2015-309579. [Epub ahead of print]

JD collected the FIT result data, determined referral status from Clinical Portal information system and clinical outcomes from Endoscopy Management System (EMS) IT system, performed statistical tests and undertook the initial data analysis, prepared the draft and final tables and figures, and wrote the Results section of the manuscript.

CM was Consultant Gastroenterologist in NHS Tayside and conceived, designed and planned the study and wrote the first draft of the manuscript. JAS was consultant in charge of the Scottish Bowel Screening Centre Laboratory and conceived, designed and planned the study and supervised the sample handling and analytical performance of the FIT and faecal calprotectin test. RW was Biomedical Scientist and analysed the faecal samples. FAC was consultant pathologist responsible for the pathology and histology in NHS Tayside. CGF (second supervisor) wrote sections of the manuscript. RJCS (first supervisor) conceived, designed and planned the study. All authors saw drafts of the paper and participated in preparation of these and approved the final version.

For the thesis chapter, JD recast the paper with a focus only on the use of FIT in the symptomatic population. The Introduction was expanded to update the review of the existing literature. Results were displayed in greater detail and included comment on gender differences in test performance and colorectal cancer site. An expanded Discussion section was also written including comment on up-to-date issues surrounding the latest update to NICE guidelines.